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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, 15/70, 15/85, 15/86, 1/21, 5/10, C07K 14/705, 16/28, A61K 38/17		A3	(11) International Publication Number: WO 98/30693
			(43) International Publication Date: 16 July 1998 (16.07.98)
(21) International Application Number: PCT/US98/00152		(74) Agents: BROOKES, A., Anders et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).	
(22) International Filing Date: 13 January 1998 (13.01.98)			
(30) Priority Data: 60/035,496 14 January 1997 (14.01.97) US 60/054,885 7 August 1997 (07.08.97) US		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).			
(72) Inventors; and		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(75) Inventors/Applicants (for US only): WEI, Ying-Fei [CN/US]; 13524 Straw Bale Lane, Darnestown, MD 20878 (US). NI, Jian [CN/US]; 5502 Manorfield Road, Rockville, MD 20853 (US). EBNER, Reinhard [DE/US]; 9906 Shelbourne Terrace #316, Gaithersburg, MD 20878 (US). YU, Guo-Liang [CN/US]; 13524 Straw Bale Lane, Darnestown, MD 20878 (US). RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Olney, MD 20832 (US). GENTZ, Reiner, L. [DE/US]; 13404 Fairland Park Drive, Silver Spring, MD 20904 (US). FENG, Ping [CN/US]; 4 Relda Court, Gaithersburg, MD 20878 (US).		(88) Date of publication of the international search report: 13 August 1998 (13.08.98)	
(54) Title: TUMOR NECROSIS FACTOR RECEPTOR 5			
(57) Abstract <p>The present invention relates to a novel human gene encoding a polypeptide which is a member of the TNF receptor family, and has now been found to bind TRAIL. More specifically, an isolated nucleic acid molecule is provided encoding a human polypeptide named tumor necrosis factor receptor-5, sometimes referred to as "TNFR-5" or "TR5", and now referred to hereinafter as "TRAIL receptor without intracellular domain" or "TRID". TRID polypeptides are also provided, as are vectors, host cells, and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists or antagonists of TRAIL polypeptide activity. Also provided are diagnostic and therapeutic methods utilizing such compositions.</p>			

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INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/US 98/00152

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/70 C12N15/85 C12N15/86 C12N1/21
C12N5/10 C07K14/705 C07K16/28 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PITTI R.M. ET AL.: "Induction of apoptosis by Apo-2L, a new member of the tumor necrosis factor cytokine family" J. BIOL. CHEM., vol. 271, no. 22, 31 May 1996, pages 12687-12690, XP002065021 cited in the application see abstract see page 12690, column 1, line 8 - line 24 ---	1-22
P,X	PAN G. ET AL.: "An antagonist decoy receptor and a death domain-containing receptor for TRAIL" SCIENCE, vol. 277, - 8 August 1997 pages 815-818, XP002065147 see the whole document ---	1-22

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☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
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- *Z* document member of the same patent family

Date of the actual completion of the international search

19 May 1998

Date of mailing of the international search report

0 3. 07. 98

Name and mailing address of the ISA

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SHERIDAN J.P. ET AL.: "Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors" SCIENCE, vol. 277, 8 August 1997, pages 818-821, XP002065023 see the whole document ---	1-22
P,X	MACFARLANE M. ET AL.: "Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL" J. BIOL. CHEM., vol. 272, no. 41, 10 October 1997, pages 25417-25420, XP002065148 see abstract see figure 1B ---	1-22
P,X	SCHNEIDER P. ET AL.: "Characterization of two receptors for TRAIL" FEBS LETTERS, vol. 416, 27 October 1997, pages 329-334, XP002065022 see the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

Int. application No.
PCT/US 98/00152

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 22 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



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Tumor Necrosis Factor Receptor 5

Field of the Invention

The present invention relates to a novel human gene encoding a polypeptide which is a member of the TNF receptor family, and has now been found to bind TRAIL. More specifically, an isolated nucleic acid molecule is provided encoding a human polypeptide named tumor necrosis factor receptor-5, sometimes referred to as "TNFR-5" or "TR5," and now referred to hereinafter as "TRAIL receptor without intracellular domain" or "TRID." TRID polypeptides are also provided, as are vectors, host cells, and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists or antagonists of TRID polypeptide activity. Also provided are diagnostic and therapeutic methods utilizing such compositions.

Related Art

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intra-cellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines, which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands, there are included TNF- α , lymphotoxin- α (LT- α , also known as TNF- β), LT- β (found in complex heterotrimer LT- α 2- β), FasL, CD40L, CD27L, CD30L, 4-IBBL, OX40L and nerve growth factor (NGF). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-IBB, OX40, low affinity p75 and NGF-receptor (Meager, A., *Biologicals* 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., *supra*).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R. *et al.*, *Nature* 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40

ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. *et al.*, *Science* 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. *et al.*, *Cell* 69:737 (1992)).

TNF and LT- α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT- α , acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT- α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., *Science* 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (p55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia *et al.*, *Cell* 74:845 (1993)).

Apoptosis, or programmed cell death, is a physiologic process essential for the normal development and homeostasis of multicellular organisms (H. Steller, *Science* 267, 1445-1449 (1995)). Derangements of apoptosis contribute to the pathogenesis of several human diseases including cancer, neurodegenerative disorders, and acquired immune deficiency syndrome (C.B. Thompson, *Science* 267, 1456-1462 (1995)). One mechanism of immune mediated killing is the engagement of death receptors. Recently, much attention has focused on the signal transduction and biological function of two cell surface death receptors, Fas/APO-1 and TNFR-1 (J.L. Cleveland *et al.*, *Cell* 81, 479-482 (1995); A. Fraser, *et al.*, *Cell* 85, 781-784 (1996); S. Nagata *et al.*, *Science* 267, 1449-56 (1995)). Both are members of the TNF receptor family which also include TNFR-2, low affinity NGFR, CD40, and CD30, among others (C.A. Smith *et al.*, *Science* 248, 1019-23 (1990); M. Tewari *et al.*, in *Modular Texts in Molecular and Cell Biology* M. Purton, Heldin, Carl, Ed. (Chapman and Hall, London, 1995)). While family members are defined by the presence of cysteine-rich repeats in their extracellular domains, Fas/APO-1 and TNFR-1 also share a region of intracellular homology, appropriately designated the "death domain", which is distantly related to the *Drosophila* suicide gene, reaper (P. Golstein, *et al.*, *Cell* 81, 185-186 (1995); K. White *et al.*, *Science* 264, 677-83 (1994)). This shared death domain suggests that both receptors interact with a related set of signal transducing molecules that, until recently, remained unidentified. Activation of Fas/APO-1 recruits the death domain-containing adapter molecule FADD/MORT1 (A.M. Chinnaiyan *et al.*, *Cell* 81, 505-12 (1995); M. P. Boldin *et al.*, *J. Biol Chem* 270, 7795-8 (1995); F.C. Kischkel *et al.*, *EMBO* 14, 5579-5588 (1995)), which in turn binds and presumably activates

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FLICE/MACH1, a member of the ICE/CED-3 family of pro-apoptotic proteases (M. Muzio *et al.*, *Cell* 85, 817-827 (1996); M.P. Boldin *et al.*, *Cell* 85, 803-815 (1996)). While the central role of Fas/APO-1 is to trigger cell death, TNFR-1 can signal an array of diverse biological activities-many of which stem from its ability to activate NF-kB (L.A. Tartaglia *et al.*, *Immunol Today* 13, 151-3 (1992)). Accordingly, TNFR-1 recruits the multivalent adapter molecule TRADD, which like FADD, also contains a death domain (H. Hsu *et al.*, *Cell* 81, 495-504 (1995); H. Hsu, *et al.*, *Cell* 84, 299-308 (1996)). Through its associations with a number of signaling molecules including FADD, TRAF2, and RIP, TRADD can signal both apoptosis and NF-kB activation (H. Hsu *et al.*, *Cell* 84, 299-308 (1996); H. Hsu, *et al.*, *Immunity* 4, 387-396 (1996)).

Recently, a new apoptosis -inducing TNF ligand has been discovered. S.R. Wiley *et al.*, *Immunity* 3,673-682 (1995) named the molecule - "TNF-related apoptosis-inducing ligand" or simply "TRAIL." The molecule has also been called "Apo-2 ligand" or "Apo-2L." R.M. Pitt *et al.*, *J. Biol. Chem.* 271,12687-12690 (1996). This molecule was also disclosed in co-pending U.S. provisional application no. 60/013,405. For convenience, the molecule will be referred to herein as TRAIL.

Unlike FAS ligand, whose transcripts appear to be largely restricted to stimulated T-cells, significant levels of TRAIL are detected in many human tissues (e.g., spleen, lung, prostate, thymus, ovary, small intestine, colon, peripheral blood lymphocytes, placenta, kidney), and is constitutively transcribed by some cell lines. It has been shown that TRAIL acts independently from the Fas ligand (Wiley *et al.*, *supra*). It has also been shown that TRAIL activates apoptosis rapidly, within a time frame that is similar to death signalling by Fas/Apo-1L, but much faster than TNF-induced apoptosis. S.A. Marsters *et al.*, *Current Biology* 6, 750-752 (1996). The inability of TRAIL to bind TNFR-1, Fas, or the recently identified DR3, suggests that TRAIL may interact with a unique receptor(s).

Several unique receptors for TRAIL have already been identified. In co-pending U.S. provisional application no. 60/035,722, DR4, a novel death domain containing receptor for TRAIL, was disclosed. See, Pan *et al.*, *Science* 276,111-113 (April 1997). The DR5 receptor, the subject of co-pending U.S. provisional patent application 60/040,846 has now been shown to bind TRAIL. In co-pending U.S. provisional patent application 60/050,936, it was predicted that the TR10 receptor would also bind TRAIL, owing to sequence homology with DR4.

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize additional novel receptors that bind TRAIL.

Summary of the Invention

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the TRID polypeptide having the amino acid sequence shown in SEQ ID NO:2, or the amino acid sequence encoded by the cDNA clone deposited as ATCC Deposit
5 Number 97798 on November 20, 1996. The nucleotide sequence determined by sequencing the deposited TRID clone, which is shown in SEQ ID NO:1 contains an open reading frame encoding a polypeptide of about 259 amino acid residues, with a leader sequence of about 26 amino acids.

The present invention also relates to recombinant vectors, which include the isolated
10 nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and methods for using them for production of TRID polypeptides or peptides by recombinant techniques.

The invention further provides an isolated TRID polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

15 The present invention also provides diagnostic assays such as quantitative and diagnostic assays for detecting levels of TRID protein. Thus, for instance, a diagnostic assay in accordance with the invention for detecting expression of TRID, or soluble form thereof, may be used to detect the ability of normal tissue to withstand or be protected from the deleterious effects of TRAIL, such as TRAIL-induced apoptosis.

20 Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes. Cellular response to TNF-family ligands include not only normal physiological responses, but also diseases associated with increased apoptosis or the inhibition of apoptosis.
25 Apoptosis - programmed cell death - is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes. Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers, autoimmune disorders, viral infections, inflammation, graft vs. host disease, acute graft rejection, and chronic graft rejection. Diseases associated
30 with increased apoptosis include AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, toxin-induced liver disease, septic shock, cachexia and anorexia.

Thus, the invention further provides a method for enhancing apoptosis induced by a TNF-family ligand, such as TRAIL, which involves administering to a cell which expresses the TRID polypeptide an effective amount of an antagonist capable of decreasing TRID's ability to
35 bind TRAIL. Preferably, TRID binding is decreased to treat a disease wherein decreased apoptosis is exhibited.

In a further aspect, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand, such as TRAIL, which involves administering to a cell an

effective amount of TRID or an agonist capable of increasing TRID activity. Preferably, TRID activity is increased to treat a disease wherein decreased apoptosis is exhibited.

Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below. Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand, such as TRAIL. The method involves contacting cells which co-expresses the TRID polypeptide and a second TNFR with a candidate compound and a TNF-family ligand (e.g., TRAIL), assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is a TRID antagonist and a decreased cellular response compared to the standard indicates that the candidate compound is TRID agonist. By the invention, a cell expressing the TNFR polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand, such as TRAIL.

Brief Description of the Figures

Figure 1 shows the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of TRID.

Figure 2 shows an alignment created by the Clustal method using the Megaline program in the DNASTar suite comparing the amino acid sequences of TNFR-5 (now called "TRID"), with other TNF receptors, as follows: TNFR1 (SEQ ID NO:3); TNFR2 (SEQ ID NO:4); NGFR (SEQ ID NO:5); LTbR (SEQ ID NO:6); FAS (SEQ ID NO:7); CD27 (SEQ ID NO:8); CD30 (SEQ ID NO:9); CD40 (SEQ ID NO:10); 4-1BB (SEQ ID NO:11); OX40 (SEQ ID NO:12); VC22 (SEQ ID NO:13); and CRMB (SEQ ID NO:14).

Figure 3 shows an analyses of the TRID amino acid sequences. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graphs, the indicate location of the highly antigenic regions of the proteins, i.e., regions from which epitope-bearing peptides of the invention may be obtained.

Figure 4 shows the nucleotide sequence of gene fragments related to the TRID gene of the present invention, including: HPRCB54R (SEQ ID NO:15), HSJAU57RA (SEQ ID NO:16), HELBP70R (SEQ ID NO:17), and HUSCB54R (SEQ ID NO:18) all of which are related to SEQ ID NO:1.

Figure 5A is an immunoblot showing that TRID-Fc (as well as DR4 and DR5) specifically bound TRAIL, but not the related cytotoxic ligand TNF α . The bottom panel of Fig.

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5A shows the input Fc-fusions present in the binding assays. **Figure 5B** is a bar graph showing that TRID-Fc blocked the ability of TRAIL to induce apoptosis. The data (mean \pm SD) shown in Fig. 5B are the percentage of apoptotic nuclei among total nuclei counted (n=4). **Figure 5C** is a bar graph showing that TRID-Fc had no effect on TNF α -induced apoptosis under conditions where TNFR1-Fc completely abolished TNF α killing.

Figure 6 is a bar graph showing that MCF7 cells expressing TRID were protected from TRAIL-induced apoptosis, as were cells expressing the virally encoded caspase inhibitor CrmA.

Detailed Description of the Preferred Embodiments

10 The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a TRID polypeptide, having the amino acid sequence shown in Figure 1 (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The nucleotide sequence shown in Figure 1 (SEQ ID NO:1) was obtained by sequencing the HPRCB54 clone, which was deposited on November 20, 1996 at the American Type Culture Collection, 12301
15 Park Lawn Drive, Rockville, Maryland 20852, and given accession number ATCC 97798. The deposited clone is inserted in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

The TRID protein of the present invention has an amino acid sequence which is 21.7% identical to and shares multiple conserved cysteine rich domains with the translation product of the human nerve growth factor (hNGF) mRNA (SEQ ID NO:5) as illustrated in Figure 2.
20 hNGF is thought to play an important role in the development, survival, apoptosis and function of neurons (Lee, F.K. et al., Cell 69:737) and lymphocytes (Torcia, M. et al., Cell 85:3369 (1996)).

Sequence alignment and comparison reveal that TRID's extracellular cysteine-rich domain to be strikingly similar to the corresponding domains of both DR4 and DR5 with 69%
25 and 52% amino acid identity, respectively. In addition, like DR4 and DR5, TRID was also found to be homologous to the cysteine-rich domain in CAR1, a chicken TNF receptor family member with amino acid identities ranging from 42-48% (J. Brojatsh et al., Cell 87: 1 (1996). A potential protective role for TRID was suggested by the finding that its transcript was detectable in many normal human tissues but not in most transformed cell lines.

30 TRID has an extracellular TRAIL binding domain and a transmembrane domain but, surprisingly, lacks a putative intracellular signalling domain, in keeping with the possibility that this receptor does not signal following ligand binding. Given the absence of an intracellular domain, this receptor was termed "TRID" for TRAIL Receptor Without an Intracellular Domain.

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc., Foster City, CA), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

Using the information provided herein, such as the nucleotide sequence set out in SEQ ID NO:1, a nucleic acid molecule of the present invention encoding a TRID polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the TRID nucleic acid molecule described in SEQ ID NO:1 was discovered in a cDNA library derived from prostate tissue. Additional clones of the same gene were also identified in cDNA libraries from the following tissues: endothelial cells, stimulated monocytes, and keratinocytes.

The determined nucleotide sequence of the TRID cDNA of SEQ ID NO:1 contains an open reading frame encoding a protein of about 259 amino acid residues, with an initiation codon at nucleotide positions 183-185 of the nucleotide sequences in Figure 1 (SEQ ID NO:1).

The open reading frame of the TRID gene shares sequence homology with the translation product of the human mRNA for NGFR, including the following conserved domains: (a) a soluble extracellular domain of about 214 amino acids (residues 27-240 of Figure 1); and (b) a transmembrane domain of about 19 amino acids (residues 241-259 of Figure 1).

As one of ordinary skill would appreciate, due to the possibility of sequencing errors discussed above, the actual complete TRID polypeptide encoded by the deposited cDNAs, which comprise about 259 amino acids, may be somewhat longer or shorter. More generally,

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the actual open reading frames may be anywhere in the range of ± 20 amino acids, more likely in the range of ± 10 amino acids, of that predicted from the first methionine codon from the N-terminus shown in SEQ ID NO:1, which is in-frame with the translated sequences shown in each respective figure. It will further be appreciated that, depending on the analytical criteria used for identifying various functional domains, the exact "address" of the extracellular and transmembrane domain(s) of the TNFR polypeptides may differ slightly from the predicted positions above. For example, the exact location of the extracellular domain in SEQ ID NO:2 may vary slightly (e.g., the address may "shift" by about 1 to about 20 residues, more likely about 1 to about 5 residues) depending on the criteria used to define the domain. In this case, the beginning of the transmembrane domain and the end of the extracellular domain were predicted on the basis of the identification of the hydrophobic amino acid sequence in the above indicated positions, as shown in Figure 5. In any event, as discussed further below, the invention further provides polypeptides having various residues deleted from the N-terminus of the complete polypeptide, including polypeptides lacking one or more amino acids from the N-terminus of the extracellular domain described herein, which constitute soluble forms of the extracellular domain of the TRID protein.

Leader and Mature Sequences

The amino acid sequence of the TRID protein includes a leader sequence and a mature protein, as shown in SEQ ID NO:2. More in particular, the present invention provides nucleic acid molecules encoding mature forms of the TRID protein. Thus, according to the signal hypothesis, once export of the growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the complete polypeptide to produce a secreted "mature" form of the protein. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding a mature TRID polypeptide having the amino acid sequence encoded by a cDNA clone identified as ATCC Deposit No. 97798. By the "mature TRID polypeptide having the amino acid sequence encoded by a cDNA clone in ATCC Deposit No. 97798" is meant the mature form(s) of the protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the deposited plasmid.

In addition, methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch

(*Virus Res.* 3:271-286 (1985)) uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje (*Nucleic Acids Res.* 14:4683-4690 (1986)) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2 where +1 indicates the amino terminus of the mature protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80% (von Heinje, *supra*). However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the complete TRID polypeptide was analyzed by a computer program "PSORT." See, K. Nakai and M. Kanehisa, *Genomics* 14:897-911 (1992). PSORT is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids -1 and 1 in SEQ ID NO:2. Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heinje. von Heinje, *supra*. Thus, the leader sequence for the TRID protein is predicted to consist of amino acid residues from about 1 to about 26, underlined in Figure 1 (corresponding to about -26 to about -1 in SEQ ID NO:2), while the mature TRID protein is predicted to consist of residues from about 1 to about 233 in SEQ ID NO:2.

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors, as well as the variability of cleavage sites for leaders in different known proteins, the mature TRID polypeptide encoded by the deposited cDNA comprises about 233 amino acids, but may be anywhere in the range of about 223 to about 243 amino acids, and the predicted leader sequence of this protein is about 26 amino acids, but may be anywhere in the range of about 16 to about 36 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA, or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA

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molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in SEQ ID NO:1; DNA molecules comprising
5 the coding sequence for the mature TRID protein; and DNA molecules which comprise a sequence substantially different from those described above, but which, due to the degeneracy of the genetic code, still encode the TRID protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

10 In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1, which have been determined from the following related cDNA clones: HELBP70R (SEQ ID NO:17), HPRCB54R (SEQ ID NO:15), HSJAU57RA (SEQ ID NO:16) and HUSCB54R (SEQ ID NO:18). The nucleotide sequences of each of these gene fragments is shown in Figure 4.

15 In another aspect, the invention provides isolated nucleic acid molecules encoding the TRID polypeptide having an amino acid sequence as encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97798. In a further embodiment, nucleic acid molecules are provided that encode the mature TRID polypeptide or the full length TRID polypeptide each lacking the N-terminal methionine.

20 The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1 or the nucleotide sequence of the TRID cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes,
25 and for detecting expression of the TRID gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in SEQ ID NO:1
30 is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-300 nt, or even 600 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA
35 or as shown in SEQ ID NO:1. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of a deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:1.

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: epitope-bearing portions of the TRID polypeptide as identified in Figure 3 and described in more detail below.

In particular, the invention provides polynucleotides having a nucleotide sequence representing the portion of SEQ ID NO:1, which consist of positions 183-959 of SEQ ID NO:1. Also contemplated are polynucleotides encoding TRID polypeptides which lack an amino terminal methionine. One such preferred polynucleotide has a nucleotide sequence representing the portion of SEQ ID NO:1 which consists of positions 186-959. Polypeptides encoded by such polynucleotides are also provided, such polypeptides comprising an amino acid sequence at positions 2-259 of SEQ ID NO:2, or the polypeptide sequence encoded by the clone deposited with the ATCC as Deposit No. 97798 lacking an amino terminal methionine.

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the TRID extracellular domain (amino acid residues from about 27 to about 240 in FIG. 1 (from about 1 to about 214 in SEQ ID NO:2)); and a polypeptide comprising the TRID transmembrane domain (amino acid residues from about 241 to about 259 in FIG. 1 (from about 215 to about 233 in SEQ ID NO:2)). Since the location of these domains have been predicted by computer graphics, one of ordinary skill would appreciate that the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to 15 residues) depending on the criteria used to define each domain.

Preferred nucleic acid fragments of the invention encode a full-length TRID polypeptide lacking the nucleotides encoding the amino-terminal methionine (e.g., nucleotides 186-959 in SEQ ID NO:1) as it is known that the methionine is cleaved naturally and such sequences maybe useful in genetically engineering TRID expression vectors. Polypeptides encoded by such polynucleotides are also contemplated by the invention.

Preferred nucleic acid fragments of the present invention further include nucleic acid molecules encoding epitope-bearing portions of the TRID protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about Gln-42 to about Glu-52 in Figure 1 (about Gln-16 to about Glu-26 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about His-58 to about Cys-66 in Figure 1 (about His-32 to about Cys-40 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Pro-68 to about Thr-76 in Figure 1 (about Pro-42 to about Thr-50 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ser-79 to about Cys-85 in Figure 1 (about Ser-53 to about Cys-59 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Cys-91 to about Thr-102 in Figure 1 (about Cys-65 to about Thr-76 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Gln-110 to about Pro-122 in Figure 1 (about Gln-84 to about Pro-96 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Arg-126 to about Val-136 in Figure 1 (about Arg-100 to about Val-110 in SEQ ID NO:2); and a polypeptide

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comprising amino acid residues from about Thr-142 to about Glu-148 in Figure 1 (about Thr-116 to about Glu-122 in SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the TRID protein. Methods for determining other such epitope-bearing portions of the TRID protein are described in detail below.

5 In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, a cDNA clone contained in ATCC Deposit No. 97798. By "stringent hybridization conditions" is intended overnight incubation at 42° C in a solution comprising: 50% formamide, 5x SSC
10 (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65° C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more
15 preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 (e.g., 50) nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide
20 (e.g., a deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:1). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the TRID cDNA shown in SEQ ID NO:1), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any
25 nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode a TRID polypeptide may include, but are not limited to the coding sequence for the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional sequences, such as
30 those encoding a leader or secretary sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing -
35 including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In

certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al.*, *Cell* 37:767-778(1984). As discussed below, other such fusion proteins include the TRID receptor fused to Fc at the N- or C- terminus.

Variant and Mutant Polynucleotides

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs, or derivatives of the TRID receptor. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the TRID polypeptide or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to: (a) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2, but lacking the amino terminal methionine; (c) a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions about 1 to about 233 in SEQ ID NO:2; (d) a nucleotide sequence encoding the polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798; (e) a nucleotide sequence encoding the mature TRID polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798; (f) a nucleotide sequence that encodes the TRID extracellular domain having the amino acid sequence at positions about 1 to about 214 in SEQ ID NO:2, or the TRID extracellular domain encoded by the cDNA contained in ATCC Deposit No. 97798; (g) a nucleotide sequence that encodes the TRID transmembrane domain having the

amino acid sequence at positions about 215 to about 233 of SEQ ID NO:2, or the TRID transmembrane domain encoded by the cDNA contained in ATCC Deposit No. 97798; and (h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), or (g) above.

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Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e), (f), (g) or (h) above. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a TRID polypeptide having an amino acid sequence in (a), (b), (c), (d), (e), (f) or (g) above.

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By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a TRID polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the TRID polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

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As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in SEQ ID NO:1, or to the nucleotide sequence of the deposited cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference

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nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence shown in SEQ ID NO:1, or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having TRID activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having TRID activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having TRID activity include, inter alia: (1) isolating a TRID gene or allelic variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the TRID gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and Northern Blot analysis for detecting TRID mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence shown in SEQ ID NO:1, or to the nucleic acid sequence of the deposited cDNA which does, in fact, encode a polypeptide having TRID receptor activity. By "a polypeptide having TRID receptor activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the TRID receptor of the invention (either the full length protein or preferably the mature protein or extracellular domain alone), as measured in a particular biological assay. The TNF family ligands (including TRAIL) induce various cellular responses by binding to TNF-family receptors, including the TRID of the present invention. Cells which express TRID are believed to have a potent cellular response to ligands including TRAIL. By a "cellular response to a TNF-family ligand" is intended any genotypic, phenotypic, and/or morphological change to a cell, cell line, tissue, tissue culture or patient that is induced by a TNF-family ligand. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with increased cell proliferation or the inhibition of increased cell proliferation, such as by the inhibition of apoptosis.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of a deposited cDNA or the nucleic acid sequence shown in SEQ ID NO:1 will encode a polypeptide "having TRID protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a

polypeptide having TRID protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

5 *Vectors and Host Cells*

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors of the invention and the production of TRID polypeptides or fragments thereof by recombinant techniques.

10 The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

15 The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for
20 translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic
25 cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and
30 conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors
35 are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods In Molecular Biology* (1986).

5 The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, peptide moieties
10 may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example,
15 EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses, it would be desirable to be able to delete
20 the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example, when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as the hIL5-receptor, have been fused with Fc portions for the purpose of high-throughput screening
25 assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition* 8:52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry* 270:16:9459-9471 (1995).

The TRID receptor can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction,
30 anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention include naturally purified products, products of
35 chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or non-glycosylated. In

addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

TRID receptor polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of TRID. Among these are applications in treatment of tumors, resistance to parasites, bacteria and viruses, to induce proliferation of T-cells, endothelial cells and certain hematopoietic cells, to treat restenosis, graft vs. host disease, to regulate anti-viral responses and to prevent certain autoimmune diseases after stimulation of TRID by an agonist. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are discussed further below.

Polypeptides and Fragments

The invention further provides an isolated TRID polypeptide having the amino acid sequences encoded by the deposited cDNA, or the amino acid sequences in SEQ ID NO:2, or a peptide or polypeptide comprising a portion of the above polypeptides.

Variant and Mutant Polypeptides

To improve or alter the characteristics of a TRID polypeptide, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins" including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

N-Terminal and C-Terminal Deletion Mutants

For instance, for many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron *et al.*, *J. Biol. Chem.*, 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing. In the present case, since the proteins of the invention are members of the TNFR polypeptide family, deletions of N-terminal amino acids up to the cysteine at position C-53 of Figure 1 (C-27 of SEQ ID NO:2) may retain some biological activity such as regulation of proliferation and apoptosis of lymphoid cells. Polypeptides having further N-terminal deletions including the C53 residue in Figure 1 (C-27 in SEQ ID NO:2), would not be expected to retain such biological activities because it is known

that these residues in a TRID-related polypeptide are required for forming a disulfide bridge to provide structural stability which is needed for ligand binding.

However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature form of the TRID protein generally will be retained when less than the majority of the residues of the complete protein or extracellular domain are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence shown in Figure 1, up to the cysteine residue in each which is at position number 53, and polynucleotides encoding such polypeptides. In particular, the present invention provides TRID polypeptides comprising the amino acid sequence of residues m-259 of Figure 1 where m is an integer in the range of 1-53 where 53 is the position of the first cysteine residue from the N-terminus of the complete TRID polypeptide (shown in Figure 1) believed to be required for activity of the TRID protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues: 1-259, 2-259, 3-259, 4-259, 5-259, 6-259, 7-259, 8-259, 9-259, 10-259, 11-259, 12-259, 13-259, 14-259, 15-259, 16-259, 17-259, 18-259, 19-259, 20-259, 21-259, 22-259, 23-259, 24-259, 25-259, 26-259, 27-259, 28-259, 29-259, 30-259, 31-259, 32-259, 33-259, 34-259, 35-259, 36-259, 37-259, 38-259, 39-259, 40-259, 41-259, 42-259, 43-259, 44-259, 45-259, 46-259, 47-259, 48-259, 49-259, 50-259, 51-259, 52-259, and 53-259 of Figure 1. Polynucleotides encoding these polypeptides also are provided.

Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, interferon gamma shows up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Döbeli et al., *J. Biotechnology* 7:199-216 (1988)). In the present case, since the protein of the invention is a member of the TNFR polypeptide family, deletions of C-terminal amino acids up to the cysteine at position 149 of Figure 1 (position 123 of SEQ ID NO:2), may retain some biological activity such as regulation of proliferation and apoptosis of lymphoid cells. Polypeptides having further C-terminal deletions including the cysteine at position 149 of Figure 1 would not be expected to retain such biological activities because it is known that this residue in TNF receptor-related polypeptides is required for forming a disulfide bridge to provide structural stability which is needed for ligand binding.

However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce

and/or bind to antibodies which recognize the complete or mature form of the protein generally will be retained when less than the majority of the residues of the complete or mature form protein are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by
5 routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of TRID shown in Figure 1 up to the cysteine at position 149 of Figure 1, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of
10 residues 1-x of the amino acid sequence in Figure 1, where x is any integer in the range of 149-259. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-x of Figure 1, where m and x are integers as described above.

15 Also included are a nucleotide sequence encoding a polypeptide consisting of a portion of a complete TRID amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 97798, where this portion excludes from 1 to about 49 amino acids from the amino terminus of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798, or from 1 to about 110 amino acids from the carboxy terminus of
20 the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

Other Mutants

In addition to terminal deletion forms of the protein discussed above, it will also be recognized by one of ordinary skill in the art that some amino acid sequences of the TRID polypeptide can be varied without significant effect on the structure or function of the proteins.

5 If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the TRID polypeptide, which show substantial TRID polypeptide activity or which include regions of TRID protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. Guidance
10 concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J. U. et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990).

Thus, the fragment, derivative, or analog of the polypeptide of SEQ ID NO:2, or that encoded by the deposited cDNA, may be: (i) one in which one or more of the amino acid
15 residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue(s), and more preferably at least one but less than ten conserved amino acid residue(s)), and such substituted amino acid residue(s) may or may not be one encoded by the genetic code; or (ii) one in which one or more of the amino acid residues includes a substituent group; or (iii) one in which the mature or soluble extracellular polypeptide
20 is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the
25 scope of those skilled in the art from the teachings herein.

Thus, the TRID of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the TRID protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro* proliferative activity.

Of particular interest are substitutions of charged amino acids with another charged amino acids and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the TRID protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al.*, *Clin Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36:838-845 (1987); Cleland *et al.* *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding of a ligand to cell surface receptors. For example, Ostade *et al.*, *Nature* 361:266-268 (1993), describes certain mutations resulting in selective binding of TNF- α to only one of the two known types of TNF receptors. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992) and de Vos *et al.* *Science* 255:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the TRID polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988).

The present inventors have discovered that the TRID polypeptide is a 259 residue protein exhibiting two main structural domains. First, the extracellular TRAIL ligand binding domain was identified within residues from about 1 to about 214 in SEQ ID NO:2. Second, the transmembrane domain was identified within residues from about 215 to about 233 in SEQ ID NO:2. As mentioned above, however, TRID, surprisingly lacks a putative intracellular signalling domain, thus, the name "TRID" (TRAIL Receptor Without an Intracellular Domain).

The polypeptides of the present invention include the polypeptide encoded by the deposited cDNA including the leader; the mature polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein); a polypeptide comprising amino acids about - 26 to about 233 in SEQ ID NO:2; a polypeptide comprising amino acids about - 25 to about 233 in SEQ ID NO:2; a polypeptide comprising amino acids about 1 to about 233 in SEQ ID NO:2; a polypeptide comprising the extracellular domain; and a polypeptide comprising the transmembrane domain; as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a TRID polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the TRID polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

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As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in SEQ ID NO:2, or to the amino acid sequence encoded by the deposited cDNA clone, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

Epitope-Bearing Portions

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998- 4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R. A. (1983) "Antibodies That React With Predetermined Sites on Proteins," *Science*, 219:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al.*, *Cell* 37:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about

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15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate TRID-specific antibodies include: a polypeptide comprising amino acid residues from about Gln-42 to about Glu-52 in Figure 1 (about Gln-16 to about Glu-26 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about His-58 to about Cys-66 in Figure 1 (about His-32 to about Cys-40 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Pro-68 to about Thr-76 in Figure 1 (about Pro-42 to about Thr-50 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Ser-79 to about Cys-85 in Figure 1 (about Ser-53 to about Cys-59 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Cys-91 to about Thr-102 in Figure 1 (about Cys-65 to about Thr-76 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Gln-110 to about Pro-122 in Figure 1 (about Gln-84 to about Pro-96 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about Arg-126 to about Val-136 in Figure 1 (about Arg-100 to about Val-110 in SEQ ID NO:2); and a polypeptide comprising amino acid residues from about Thr-142 to about Glu-148 in Figure 1 (about Thr-116 to about Glu-122 in SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the TRID protein.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. See, e.g., Houghten, R. A. (1985) "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids." *Proc. Natl. Acad. Sci. USA* 82:5131-5135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe *et al.*, *supra*; Wilson *et al.*, *supra*; Chow, M. *et al.*, *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle, F. J. *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985). Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. See, for instance, Geysen *et al.*, *supra*. Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. *et al.* (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and

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libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

5 **Fusion Proteins**

As one of skill in the art will appreciate, TRID receptor polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has
10 been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker et al., *Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be
15 more efficient in binding and neutralizing other molecules than the monomeric TRID protein or protein fragment alone (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)).

Antibodies

TRID-protein specific antibodies for use in the present invention can be raised against the intact TRID proteins or an antigenic polypeptide fragment thereof, which may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or
20 mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')₂ fragments) which are capable of specifically binding to a TNFR protein. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and
25 may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)). Thus, these fragments are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the TRID protein or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal
30 antibodies. In a preferred method, a preparation of TRID protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies. Such monoclonal antibodies can be prepared using hybridoma technology (Köhler
35 et al., *Nature* 256:495 (1975); Köhler et al., *Eur. J. Immunol.* 6:511 (1976); Köhler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell*

Hybridomas, Elsevier, N.Y., (1981) pp. 563-681). In general, such procedures involve immunizing an animal (preferably a mouse) with a TRID protein antigen or, more preferably, with a TRID protein-expressing cell. Suitable cells can be recognized by their capacity to bind anti-TRID protein antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56° C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the desired TRID antigen.

Alternatively, additional antibodies capable of binding to the TRID antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, TRID-protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the TRID protein-specific antibody can be blocked by the TRID protein antigen. Such antibodies comprise anti-idiotypic antibodies to the TRID protein-specific antibody and can be used to immunize an animal to induce formation of further TRID protein-specific antibodies.

It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). Alternatively, TRID protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For *in vivo* use of anti-TRID in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., *Nature* 312:643 (1984); Neuberger et al., *Nature* 314:268 (1985).

Immune System-Related Disorders

Diagnosis

The present inventors have discovered that TRID is expressed in hematopoietic tissues and other normal human tissues. For a number of immune system-related disorders, substantially altered (increased or decreased) levels of TRID gene expression can be detected in immune system tissue or other cells or bodily fluids (e.g., sera and plasma) taken from an individual having such a disorder, relative to a "standard" TRID gene expression level, that is, the TRID expression level in immune system tissues or bodily fluids from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of an immune system disorder, which involves measuring the expression level of the gene encoding the TRID protein in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard TRID gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

In particular, it is believed that certain tissues in mammals with cancer express significantly enhanced levels of the TRID protein and mRNA encoding the TRID when compared to a corresponding "standard" level. Further, it is believed that enhanced levels of the TRID protein can be detected in certain body fluids (e.g., sera and plasma) from mammals with such a cancer when compared to sera from mammals of the same species not having the cancer.

Thus, the invention provides a diagnostic method useful during diagnosis of an immune system disorder, including cancers which involves measuring the expression level of the gene encoding the TRID protein in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard TRID gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

Where a diagnosis of a disorder in the immune system including diagnosis of a tumor has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting altered (particularly enhanced) gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "assaying the expression level of the gene encoding a TRID protein" is intended qualitatively or quantitatively measuring or estimating the level of TRID or the level of the mRNA encoding TRID in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the TRID protein level or mRNA level in a second biological sample). Preferably, the TRID protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard TRID protein level or mRNA level, the standard being taken from a second biological

sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the immune system. As will be appreciated in the art, once standard TRID protein levels or mRNA levels are known, they can be used repeatedly as a standard for comparison.

5 By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains TRID protein or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free extracellular domain(s) (or soluble form(s)) of a TRID protein, immune system tissue, and other tissue sources found to express complete or
10 extracellular domain of TRID. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The invention also contemplates the use of a gene of the present invention for diagnosing mutations in the TRID gene. For example, if a mutation is present in one of the
15 genes of the present invention, conditions would result from a lack of production of the receptor polypeptides of the present invention. Further, mutations which enhance receptor polypeptide activity would lead to diseases associated with an over expression of the receptor polypeptide, e.g., endotoxic shock. Mutations in the genes can be detected by comparing the sequence of the defective gene with that of a normal one. Subsequently one can verify that a
20 mutant gene is associated with a disease condition or the susceptibility to a disease condition. That is, a mutant gene which leads to the overexpression of TRID would be associated with an inability of TRAIL to inhibit tumor growth.

Other immune system disorders which may be diagnosed by the foregoing assays include hypersensitivity, allergy, infectious disease, graft-host disease, immunodeficiency,
25 autoimmune diseases and the like.

Individuals carrying mutations in the genes of the present invention may be detected at the DNA level by a variety of techniques. Nucleic acids used for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva and tissue biopsy among other tissues. The genomic DNA may be used directly for detection or may be amplified enzymatically by
30 using PCR (Saiki et al., Nature, 324:163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid of the instant invention can be used to identify and analyze mutations in the human genes of the present invention. For example, deletions and insertions can be detected by a change in the size of the amplified product in comparison to the normal genotype. Point mutations can be
35 identified by hybridizing amplified DNA to radiolabeled RNA or alternatively, radiolabeled antisense DNA sequences of the present invention. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures. Such a diagnostic would be particularly useful for prenatal or even neonatal testing.

Sequence differences between the reference gene and "mutants" may be revealed by the direct DNA sequencing method. In addition, cloned DNA segments may be used as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primary used with double stranded PCR product or a single stranded template molecule generated by a modified PCR product. The sequence determination is performed by conventional procedures with radiolabeled nucleotides or by automatic sequencing procedures with fluorescent tags.

Sequence changes at the specific locations may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (for example, Cotton et al., PNAS, 85:4397-4401 (1985)).

Assaying TRID protein levels in a biological sample can occur using antibody-based techniques. For example, TRID protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M., et al., *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting TRID gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium ($^{99\text{m}}\text{Tc}$), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying TRID protein levels in a biological sample obtained from an individual, TRID proteins can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of TRID proteins include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A TRID-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain TRID protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in

Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Treatment

5 The Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (Goeddel, D.V. *et al.*, "Tumor Necrosis Factors: Gene Structure and Biological Activities," *Symp. Quant. Biol.* 51:597-609 (1986), Cold Spring Harbor; Beutler, B., and
10 Cerami, A., *Annu. Rev. Biochem.* 57:505-518 (1988); Old, L.J., *Sci. Am.* 258:59-75 (1988); Fiers, W., *FEBS Lett.* 285:199-224 (1991)). The TNF-family ligands induce such various cellular responses by binding to TNF-family receptors. Cells which express a TRID polypeptide and have a potent cellular response to TNFR ligands include lymphocytes, endothelial cells, keratinocytes, and prostate tissue. By "a cellular response to a TNF-family
15 ligand" is intended any genotypic, phenotypic, and/or morphologic change to a cell, cell line, tissue, tissue culture or patient that is induced by a TNF-family ligand. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with increased apoptosis or the inhibition of apoptosis.

 Diseases associated with increased cell survival, or the inhibition of apoptosis, include
20 cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, such as breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as systemic lupus erythematosus and immune-related glomerulonephritis rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), information graft v. host disease, acute graft rejection, and chronic
25 graft rejection. Diseases associated with increased apoptosis include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration); myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and
30 anorexia.

 Thus, in one aspect, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses a TNFR polypeptide an effective amount of an antagonist of the TRID polypeptide, capable of inhibiting TRID expression or its ligand binding ability (e.g., to TRAIL).
35 Preferably, TNFR mediated signaling is increased to treat a disease wherein decreased apoptosis is exhibited. Antagonist can include monoclonal antibodies directed against the TRID polypeptide.

By "antagonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating apoptosis. By "agonist" is intended naturally occurring and synthetic compounds capable of inhibiting apoptosis. Whether any candidate "antagonist" or "agonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known
5 TNF-family ligand/receptor cellular response assays, including those described in more detail below.

One such screening procedure involves the use of melanophores which are transfected to co-express a TNFR receptor which binds a TRAIL such as DR4 or DR5, described elsewhere herein, and the TRID receptor of the present invention. Such a screening technique
10 is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed, for example, for screening for a compound which inhibits (or enhances) the activity of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptors with both a TNF-family ligand and the candidate antagonist (or agonist). Inhibition or enhancement of the signal generated by the ligand indicates that the compound is
15 an antagonist or agonist of TRID activity. The TRID polypeptide and its agonists inhibit activation of the TNFR receptor, e.g., TRAIL receptor, whereas antagonists will increase activation.

Other screening techniques include the use of cells which express a TRAIL receptor and TRID (for example, transfected CHO cells) in a system which measures extracellular pH
20 changes caused by receptor activation, for example, as described in *Science* 246:181-296 (October 1989). For example, compounds may be contacted with a cell which expresses a TRAIL receptor polypeptide and TRID of the present invention and a second messenger response, e.g., signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the TRAIL receptor.

Another such screening technique involves introducing RNA encoding the receptors
25 into *Xenopus* oocytes to transiently express TRID and a TRAIL receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing in cells a construct wherein the
30 TRAIL receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase signal in the presence of TRID either co-expressed or added in
35 soluble form along with the candidate compound.

Another method involves screening for compounds which inhibit activation of a TRAIL receptor polypeptide in the presence of the TRID polypeptide of the present invention, either co-expressed or in soluble form. Agonists of the present invention are identified by

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determining inhibition of binding of labeled ligand to cells which have the TRAIL receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding a TRAIL binding receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labelled TRAIL and TRID. TRAIL can be
5 labeled, e.g., by radioactivity. The amount of labeled TRAIL bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the TRID receptor as determined by an increase of labeled TRAIL which binds to the TRAIL receptor, the compound is a TRID antagonist.

Further screening assays for agonist and antagonist of the present invention are
10 described in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem.* 267(7):4304-4307(1992).

Thus, in a further aspect, a screening method is provided for determining whether a candidate TRID antagonist or agonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand (e.g., apoptosis induced by TRAIL). The method involves contacting cells which express a TNFR polypeptide with a candidate compound, TRID, and a TNF-family
15 ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in the presence of TRID but in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an antagonist and a decreased cellular response compared to the standard indicates that the candidate compound is an agonist. By
20 "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or a TNF-family ligand (e.g., determining or estimating an increase or decrease in T cell proliferation or tritiated thymidine labeling). By the invention, a cell expressing the TNFR polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

Antagonist according to the present invention include naturally occurring and synthetic
25 compounds such as, for example, TNF family ligand peptide fragments, transforming growth factor, neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor suppressors (p53), cytolytic T cells and antimetabolites. Preferred agonist include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine
30 arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and - amyloid peptide. (*Science* 267:1457-1458 (1995)). Further preferred antagonist includes polyclonal and monoclonal antibodies raised against the TRID polypeptide, or a fragment thereof.

Agonists according to the present invention include naturally occurring and synthetic
35 compounds such as, for example, the CD40 ligand, neutral amino acids, zinc, estrogen, androgens, viral genes (such as Adenovirus *E1B*, Baculovirus *p35* and *IAP*, Cowpox virus *crmA*, Epstein-Barr virus *BHRF1*, *LMP-1*, African swine fever virus *LMW5-HL*, and Herpesvirus *yl 34.5*), calpain inhibitors, cysteine protease inhibitors, and tumor promoters

(such as PMA, Phenobarbital, and γ -Hexachlorocyclohexane). Other Agonists include polyclonal and monoclonal antagonist antibodies raised against TRAIL polypeptides or a fragment thereof.

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem.* 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee *et al.*, *Nucleic Acids Research* 6:3073 (1979); Cooney *et al.*, *Science* 241:456 (1988); and Dervan *et al.*, *Science* 251:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into receptor polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the receptor.

Further agonist according to the present invention include soluble forms of TRID, i.e., TRID fragments that include the ligand binding domain from the extracellular region of the full-length receptor. Such soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize TNFR mediated signaling by competing with the cell surface TNFR for binding to TNF-family ligands. Thus, soluble forms of the TRID receptor that include the ligand binding domain are novel cytokines capable of inhibiting apoptosis induced by TNF-family ligands. Other such cytokines are known in the art and include Fas B (a soluble form of the mouse Fas receptor) that acts physiologically to limit apoptosis induced by Fas ligand (Hughes, D.P. and Crispe, I.N., *J. Exp. Med.* 182:1395-1401 (1995)).

As indicated polyclonal and monoclonal antibody agonist or antagonist according to the present invention can be raised according to the methods disclosed in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem.* 267(7):4304-4307(1992); Tartaglia, L.A. *et al.*, *Cell* 73:213-216 (1993), and PCT Application WO 94/09137. The term "antibody" (Ab) or "monoclonal antibody" (mAb) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and F(ab')₂ fragments) which are capable of binding an antigen. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al.*, *J. Nucl. Med.* 24:316-325 (1983)).

Antibodies according to the present invention may be prepared by any of a variety of methods described above, and known in the art

Proteins and other compounds which bind the extracellular domains are also candidate agonist and antagonist according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature* 340:245-246 (1989)). A modified version of the yeast two-hybrid system has been described by Roger Brent and his colleagues (Gyuris, J. *et al.*, *Cell* 75:791-803 (1993); Zervos, A.S. *et al.*, *Cell* 72:223-232 (1993)).

By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, TRID ligands, TRAIL, TNF- α , lymphotoxin- α (LT- α , also known as TNF- β), LT- β , FasL, CD40, CD27, CD30, 4-1BB, OX40 and nerve growth factor (NGF). Experiments concerning the ability of TRID to bind TRAIL are described below in Example 5.

Representative therapeutic applications of the present invention are discussed in more detail below. The state of immunodeficiency that defines AIDS is secondary to a decrease in the number and function of CD4⁺ T-lymphocytes. Recent reports estimate the daily loss of CD4⁺ T cells to be between 3.5×10^7 and 2×10^9 cells (Wei X., *et al.*, *Nature* 373:117-122 (1995)). One cause of CD4⁺ T cell depletion in the setting of HIV infection is believed to be HIV-induced apoptosis. Indeed, HIV-induced apoptotic cell death has been demonstrated not only *in vitro* but also, more importantly, in infected individuals (Ameisen, J.C., *AIDS* 8:1197-1213 (1994); Finkel, T.H., and Banda, N.K., *Curr. Opin. Immunol.* 6:605-615(1995); Muro-Cacho, C.A. *et al.*, *J. Immunol.* 154:5555-5566 (1995)). Furthermore, apoptosis and CD4⁺ T-lymphocyte depletion is tightly correlated in different animal models of AIDS (Brunner, T., *et al.*, *Nature* 373:441-444 (1995); Gougeon, M.L., *et al.*, *AIDS Res. Hum. Retroviruses* 9:553-563 (1993)) and, apoptosis is not observed in those animal models in which viral replication does not result in AIDS (Gougeon, M.L. *et al.*, *AIDS Res. Hum. Retroviruses* 9:553-563 (1993)). Further data indicates that uninfected but primed or activated T lymphocytes from HIV-infected individuals undergo apoptosis after encountering the TNF-family ligand FasL. Using monocytic cell lines that result in death following HIV infection, it has been demonstrated that infection of U937 cells with HIV results in the *de novo* expression of FasL and that FasL mediates HIV-induced apoptosis (Badley, A.D. *et al.*, *J. Virol.* 70:199-206 (1996)). Further the TNF-family ligand was detectable in uninfected macrophages and its expression was upregulated following HIV infection resulting in selective killing of uninfected CD4 T-lymphocytes (Badley, A.D. *et al.*, *J. Virol.* 70:199-206 (1996)). Thus, by the invention, a method for treating HIV⁺ individuals is provided which involves administering soluble TRID (e.g., the extracellular domain) or an agonist of the present invention to reduce

selective killing of CD4 T-lymphocytes. Modes of administration and dosages are discussed in detail below.

In rejection of an allograft, the immune system of the recipient animal has not previously been primed to respond because the immune system for the most part is only primed by environmental antigens. Tissues from other members of the same species have not been presented in the same way that, for example, viruses and bacteria have been presented. In the case of allograft rejection, immunosuppressive regimens are designed to prevent the immune system from reaching the effector stage. However, the immune profile of xenograft rejection may resemble disease recurrence more than allograft rejection. In the case of disease recurrence, the immune system has already been activated, as evidenced by destruction of the native islet cells. Therefore, in disease recurrence the immune system is already at the effector stage. Antagonist of the present invention are able to suppress the immune response to both allografts and xenografts because lymphocytes activated and differentiated into effector cells will express TNFR polypeptides, and thereby are susceptible to compounds which enhance TNFR activity. Thus, the present invention further provides a method for creating immune privileged tissues. Agonist of the invention can further be used in the treatment of Inflammatory Bowel-Disease.

Formulations

The TRID polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with TRID polypeptide alone), the site of delivery of the TRID polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of TRID polypeptide for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of TRID polypeptide administered parenterally per dose will be in the range of about 1 $\mu\text{g/kg/day}$ to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day , and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the TRID polypeptide is typically administered at a dose rate of about 1 $\mu\text{g/kg/hour}$ to about 50 $\mu\text{g/kg/hour}$, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the TRID of the invention may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler,

diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The TRID polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., *Id.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release TRID polypeptide compositions also include liposomally entrapped TRID polypeptides. Liposomes containing TRID polypeptides are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. (USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal TNFR polypeptide therapy.

For parenteral administration, in one embodiment, the TRID polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the TRID polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose

or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

5 The TRID polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of TRID polypeptide salts.

10 TRID polypeptides to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic TRID polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

15 TRID polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous TRID polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized TRID polypeptide using bacteriostatic Water-for-Injection.

20 The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in
25 conjunction with other therapeutic compounds.

Chromosome Assays

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for
30 identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

35 In certain preferred embodiments in this regard, the cDNAs herein disclosed are used to clone genomic DNA of a TRID protein gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1: Expression and Purification of the "His-tagged" Extracellular form of TRID in E. coli

The bacterial expression vector pQE9 (pD10) is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE9 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino terminus of that polypeptide.

The DNA sequence encoding the desired portion of the TRID protein comprising the extracellular form of the TRID amino acid sequence is amplified from the deposited cDNA

clone using PCR oligonucleotide primers which anneal to sequence encoding the amino terminal sequences of the desired portion of the TRID protein and to carboxy terminal sequences of the desired portion of the extracellular form of the TRID protein in the deposited cDNA. Additional nucleotides containing restriction sites to facilitate cloning in the pQE9
5 vector are added to the 5' and 3' primer sequences, respectively.

For cloning the extracellular form of the TRID protein, the 5' primer has the sequence 5' CGCGGATCCACCACTGCCCCGGCAGGAG 3' (SEQ ID NO: 19) containing the underlined BamHI restriction site followed by 18 nucleotides of the amino terminal coding sequence of the extracellular TRID sequence in SEQ ID NO:2. One of ordinary skill in the art
10 would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins and where the 3' primer ends may be varied to amplify a DNA segment encoding any desired portion of the complete TRID protein shorter or longer than the extracellular form of the protein. The 3' primer has the sequence 5' GCGTCTAGACTAGTAATGAGAAGAGGCAGG 3' (SEQ ID NO:20) containing the
15 underlined XbaI restriction site followed by 18 nucleotides complementary to the 3' end of cDNA encoding the extracellular domain of the TRID protein in Figure 1.

The amplified TRID DNA fragment and the vector pQE9 are digested with BamHI and XbaI and the digested DNAs are then ligated together. Insertion of the TRID DNA into the restricted pQE9 vector places the TRID protein coding region downstream from the IPTG-
20 inducible promoter and in-frame with an initiating AUG and the six histidine codons.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook et al., *Molecular Cloning: a Laboratory Manual*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac
25 repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing TRID protein, is available commercially from QIAGEN, Inc., *supra*. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned
30 DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-β-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM
35 to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI

40

repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4° C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the TRID is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc.,
5 *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist, 1995, QIAGEN, Inc., *supra*). Briefly the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then
10 washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the TRID is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions
15 are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins can be eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4°
20 C or frozen at -80° C.

Example 2: Cloning and Expression of TRID in a Baculovirus Expression System

In this illustrative example, the plasmid shuttle vector pA2 is used to insert the cloned DNA encoding complete protein, including its naturally associated secretory signal (leader)
25 sequence, into a baculovirus to express the mature TRID protein, using standard methods as described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI,
30 Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with
35 wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

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Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

The cDNA sequence encoding the full length TRID protein in a deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in SEQ ID NO:2 is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer for TRID has the sequence 5' CGCTCTAGACCGCCATCATGGCCCGGATCCCCAAG 3' (SEQ ID NO:21) containing the underlined XbaI restriction enzyme site. The described primers encode an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol.* 196:947-950 (1987). The 3' primer for TRID has the sequence 5' GCGTCTAGACTAGTAATGAGAAGAGGCAGG 3' (SEQ ID NO:22) containing the underlined XbaI restriction site.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with the appropriate restriction enzyme for each of the primers used, as specified above, and again is purified on a 1% agarose gel.

The plasmid is digested with the same restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Statagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human TNF receptor gene by digesting DNA from individual colonies using the enzymes used immediately above and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pA2-TRID.

Five µg of the plasmid pA2-TRID is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA* 84: 7413-7417 (1987). One µg of BaculoGold™ virus DNA and 5 µg of the plasmid pA2-TNFR are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin

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plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days, the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 µl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later, the supernatants of these culture dishes are harvested and then they are stored at 4° C. The recombinant virus is called V-TRID.

To verify the expression of the V-TRID, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-TRID at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 µCi of ³⁵S-methionine and 5 µCi ³⁵S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the mature protein and thus the cleavage point and length of the secretory signal peptide.

Example 3: Cloning and Expression of TRID in Mammalian Cells

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late

promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include: human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The dihydrofolate reductase (DHFR) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J.* 227:277-279 (1991); Bebbington et al., *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

The expression plasmid, pTRID-HA, is made by cloning a cDNA encoding TRID into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/Amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the

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SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al.*, *Cell* 37: 767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the TRID is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The TRID cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of a TNF receptor in *E. coli*. Suitable primers include the following, which are used in this example. The 5' primer for TNFR-5, containing the underlined EcoRI site, has the following sequence: 5' CGCGAATTCGCCATCATGGCCCGGATCCCCAAG 3' (SEQ ID NO:23). The 3' primer, containing the underlined XbaI site, has the following sequence: 5' GCGTCTAGAGTAATGAGAAGAGGCAGG 3' (SEQ ID NO:24).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with XbaI and EcoRI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the fragment encoding the TRID polypeptide.

For expression of recombinant TRID, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al.*, *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of TRID by the vector.

Expression of the pTRID-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow *et al.*, *Antibodies: A Laboratory Manual*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ³⁵S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE

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and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of TRID polypeptides. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J. Biol. Chem.* 253:1357-1370, Hamlin, J. L. and Ma, C. 1990, *Biochem. et Biophys. Acta*, 1097:107-143, Page, M. J. and Sydenham, M. A. 1991, *Biotechnology* 9:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology* 5:438-447 (1985) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart et al., *Cell* 41:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, Xba I, and Asp718. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human β -actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the TRID receptor polypeptide in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA* 89:5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

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The plasmid pC4 is digested with the restriction enzymes appropriate for the specific primers used to amplify TRID as outlined below and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

5 The DNA sequence encoding the TRID polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the desired portion of the gene. The 5' primer for TRID containing the underlined XbaI site, has the following sequence: 5' CGCTCTAGACCGCCATCATGGCCCGGATCCCCAAG 3' (SEQ ID NO:25).

10 The 3' primer for TRID, containing the underlined XbaI site, has the following sequence: 5' GCGTCTAGACTAGTAATGAGAAGAGGCAGG 3' (SEQ ID NO:26).

15 The amplified fragment is digested with the endonucleases which will cut at the engineered restriction site(s) and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

20 Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. Five µg of the expression plasmid pC4 is cotransfected with 0.5 µg of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days, single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 µM, 2 µM, 5 µM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 µM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 4: Tissue distribution of TRID mRNA expression

35 Northern blot analysis was carried out to examine TRID gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the TRID protein (SEQ ID NO:1) was labeled with ³²P using the *rediprime*TM DNA labeling system (Amersham Life Science),

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according to manufacturer's instructions. After labeling, the probe was purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe was then used to examine various human tissues for TRID mRNA.

5 Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) were obtained from Clontech (Palo Alto, CA) and examined with labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots were mounted and exposed to film at -70°C overnight. The films were developed according to standard procedures. Expression of TRID was detected in many normal human tissues, such as heart, brain, placenta, lung, liver, kidney, pancreas, spleen, thymus, peripheral blood leukocytes (PBLs), lymph node, bone marrow, and fetal liver, but not in most transformed cancer cell lines.

10 Expression of TRID was also assessed by Northern blot in the following cancer cell lines, HL60 (promyelocytic leukemia), Hela cell S3, K562 (chronic myelogenous leukemia), MOLT4 (lymphoblast leukemia), Raji (Burkitt's lymphoma), SW480 (colorectal adenocarcinoma), A549 (lung carcinoma), and G361 (melanoma), and was detected in only SW480 and Hela cell S3.

20 *Example 5*

The Extracellular Domain of TRID Binds the Cytotoxic Ligand--TRAIL, Blocks TRAIL-Induced Apoptosis

25 As discussed above, TRAIL/Apo2L is a cytotoxic ligand that belongs to the tumor necrosis factor (TNF) ligand family and induces rapid cell death of many transformed cell lines, but not normal tissues, despite its death domain containing receptor, DR4, being expressed on both cell types. This example identifies an antagonist decoy receptor, designated "TRAIL Receptor Without Intracellular Domain" or "TRID", that also binds TRAIL and may in part explain the resistant phenotype of normal tissues. That is, TRID, an antagonistic receptor, binds and sequesters TRAIL, but is incapable of transducing an intracellular signal.

30 Given the similarity of the extracellular ligand binding cysteine-rich domains of TRID and DR4, the present inventors theorized that TRID would also bind TRAIL. To confirm this, the soluble extracellular ligand binding domain of TRID was expressed as a fusion to the Fc portion of human immunoglobulin (IgG).

35 As shown in Fig. 5a, TRID-Fc specifically bound TRAIL, but not the related cytotoxic ligand TNFα. In this experiment, the Fc-extracellular domains of TRID, DR5, DR4, or TNFR1 and the corresponding ligands were prepared and binding assays performed as described in Pan *et al.*, *Science* 276:111 (1997). The respective Fc-fusions were precipitated with protein G-Sepharose and co-precipitated soluble ligands were detected by immunoblotting

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with anti-Flag (Babco) or anti-myc-HRP (BMB). The bottom panel of Fig. 5A shows the input Fc-fusions present in the binding assays.

Additionally, TRID-Fc blocked the ability of TRAIL to induce apoptosis (Fig. 5B). MCF7 cells were treated with soluble TRAIL (200 ng/ml) in the presence of equal amounts of Fc-fusions or Fc alone. Six hours later, cells were fixed and examined as described in Pan et al., *Id.* The data (mean \pm SD) shown in Fig. 5B are the percentage of apoptotic nuclei among total nuclei counted (n=4).

Further, TRID-Fc had no effect on TNF α -induced apoptosis under conditions where TNFR1-Fc completely abolished TNF α killing (Fig 5C). MCF7 cells were treated with TNF α (40 ng/ml; Genentech, Inc.) in the presence of equal amounts of Fc-fusions or Fc alone. Nuclei were stained and examined 11-15 hours later.

Example 6

TRID Protects Cells from TRAIL-Induced Apoptosis

As shown in Fig. 6, cells expressing TRID were protected from TRAIL-induced apoptosis as were cells expressing the virally encoded caspase inhibitor CrmA.

Given the absence of an intracellular signalling domain, it was likely that native TRID could itself similarly attenuate TRAIL-induced cell death. This was confirmed by asking if overexpression of native TRID in TRAIL-sensitive cells (MCF7) would protect them from TRAIL-induced apoptosis. Overexpression of TRID by itself did not induce apoptosis. However, when the cells were exposed to TRAIL, cells expressing TRID were as protected from TRAIL-induced apoptosis as were cells expressing the virally encoded caspase inhibitor CrmA (Fig. 6).

MCF7 cells were transfected with TRID, or CrmA expression construct or vector alone together with a b-Gal reporter construct. Twenty four hours after transfection, TRAIL was added at 50 ng/ml and 100 ng/ml. Six hours later, cells were stained with X-gal as previously described (A.M. Chinnaiyan, *et al.*, *Cell* 81, 505-12 (1995); M.P. Boldin, *et al.*, *J Biol Chem* 270, 7795-8 (1995); F.C. Kischkel, *et al.*, *EMBO* 14, 5579-5588 (1995)), and examined microscopically.

Taken together, these findings are consistent with a guardian role for TRID that allows normal tissues to withstand the potentially deleterious effects of constitutively expressed TRAIL.

The new identification of the antagonist decoy receptor TRID as a receptor for TRAIL adds further complexity to the biology of TRAIL-initiated signal transduction.

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It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

- 5 The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: WEI, YING-FEI, et al.
- (ii) TITLE OF INVENTION: TUMOR NECROSIS FACTOR RECEPTOR 5
- (iii) NUMBER OF SEQUENCES: 26
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: HUMAN GENOME SCIENCES, INC.
 - (B) STREET: 9410 KEY WEST AVENUE
 - (C) CITY: ROCKVILLE
 - (D) STATE: MD
 - (E) COUNTRY: US
 - (F) ZIP: 20850
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: BROOKES, ANDERS A
 - (B) REGISTRATION NUMBER: 36,373
 - (C) REFERENCE/DOCKET NUMBER: PF341
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (301) 309-8504
 - (B) TELEFAX: (301) 309-8512

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1392 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 183..959

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTCTCCACG CGCACGAAC T CAGCCAACGA TTTCTGATAG ATTTTTTGGGA GTTTGACCAG

60

57

AGATGCAAGG GGTGAAGGAG CGCTTCCTAC CGTTAGGGAA CTCTGGGGAC AGAGCGCCCC	120
GGCCGCCTGA TGGCCGAGGC AGGGTGCGAC CCAGGACCCA GGACGGCGTC GGAACCATTA	180
CC ATG GCC CGG ATC CCC AAG ACC CTA AAG TTC GTC GTC GTC ATC GTC Met Ala Arg Ile Pro Lys Thr Leu Lys Phe Val Val Val Ile Val -26 -25 -20 -15	227
GCG GTC CTG CTG CCA GTC CTA GCT TAC TCT GCC ACC ACT GCC CGG CAG Ala Val Leu Leu Pro Val Leu Ala Tyr Ser Ala Thr Thr Ala Arg Gln -10 -5 -1 1 5	275
GAG GAA GTT CCC CAG CAG ACA GTG GCC CCA CAG CAA CAG AGG CAC AGC Glu Glu Val Pro Gln Gln Thr Val Ala Pro Gln Gln Gln Arg His Ser 10 15 20	323
TTC AAG GGG GAG GAG TGT CCA GCA GGA TCT CAT AGA TCA GAA CAT ACT Phe Lys Gly Glu Glu Cys Pro Ala Gly Ser His Arg Ser Glu His Thr 25 30 35	371
GGA GCC TGT AAC CCG TGC ACA GAG GGT GTG GAT TAC ACC AAC GCT TCC Gly Ala Cys Asn Pro Cys Thr Glu Gly Val Asp Tyr Thr Asn Ala Ser 40 45 50	419
AAC AAT GAA CCT TCT TGC TTC CCA TGT ACA GTT TGT AAA TCA GAT CAA Asn Asn Glu Pro Ser Cys Phe Pro Cys Thr Val Cys Lys Ser Asp Gln 55 60 65	467
AAA CAT AAA AGT TCC TGC ACC ATG ACC AGA GAC ACA GTG TGT CAG TGT Lys His Lys Ser Ser Cys Thr Met Thr Arg Asp Thr Val Cys Gln Cys 70 75 80	515
AAA GAA GGC ACC TTC CGG AAT GAA AAC TCC CCA GAG ATG TGC CGG AAG Lys Glu Gly Thr Phe Arg Asn Glu Asn Ser Pro Glu Met Cys Arg Lys 85 90 95	563
TGT AGC AGG TGC CCT AGT GGG GAA GTC CAA GTC AGT AAT TGT ACG TCC Cys Ser Arg Cys Pro Ser Gly Glu Val Gln Val Ser Asn Cys Thr Ser 100 105 110	611
TGG GAT GAT ATC CAG TGT GTT GAA GAA TTT GGT GCC AAT GCC ACT GTG Trp Asp Asp Ile Gln Cys Val Glu Glu Phe Gly Ala Asn Ala Thr Val 115 120 125	659
GAA ACC CCA GCT GCT GAA GAG ACA ATG AAC ACC AGC CCG GGG ACT CCT Glu Thr Pro Ala Ala Glu Glu Thr Met Asn Thr Ser Pro Gly Thr Pro 130 135 140	707
GCC CCA GCT GCT GAA GAG ACA ATG AAC ACC AGC CCA GGG ACT CCT GCC Ala Pro Ala Ala Glu Glu Thr Met Asn Thr Ser Pro Gly Thr Pro Ala 145 150 155	755
CCA GCT GCT GAA GAG ACA ATG ACC ACC AGC CCG GGG ACT CCT GCC CCA Pro Ala Ala Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Pro 160 165 170	803
GCT GCT GAA GAG ACA ATG ACC ACC AGC CCG GGG ACT CCT GCC CCA GCT Ala Ala Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Pro Ala 175 180 185	851
GCT GAA GAG ACA ATG ACC ACC AGC CCG GGG ACT CCT GCC TCT TCT CAT Ala Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Ser Ser His	899

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190	195	200	
TAC CTC TCA TGC ACC ATC GTA GGG ATC ATA GPT CTA ATT GTG CTT CTG			947
Tyr Leu Ser Cys Thr Ile Val Gly Ile Ile Val Leu Ile Val Leu Leu			
205	210	215	
ATT GTG TTT GTT TGAAAGACTT CACTGTGGAA GAAATTCCTT CCTTACCTGA			999
Ile Val Phe Val			
220			
AAGGTTTCAGG TAGGCGCTGG CTGAGGGCGG GGGGCGCTGG ACACTCTCTG CCCTGCCTCC			1059
CTCTGCTGTG TTCCACAGA CAGAAACGCC TGGCCCTGCC CCAAGTCCTG GTGTCTCCAG			1119
CCTGGCTCTA TCTTCTCTCT TGTGATCGTC CCATCCCCAC ATCCCGTGCA CCCCCAGGA			1179
CCCTGGTCTC ATCAGTCCCT CTCCTGGAGC TGGGGGTCCA CACATCTCCC AGCCAAGTCC			1239
AAGAGGCAGG GCCAGTTCCT CCCATCTTCA GGCCAGCCA GGCAGGGGGC AGTCGGCTCC			1299
TCAACTGGGT GACAAGGGTG AGGATGAGAA GTGGTCACGG GATTTATTCA GCCTTGGTCA			1359
GAGCAGAACA CAGAGATTTT CCGTGAAAAA AAA			1392

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 259 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ala	Arg	Ile	Pro	Lys	Thr	Leu	Lys	Phe	Val	Val	Val	Ile	Val	Ala
1				5				10					15		
Val	Leu	Leu	Pro	Val	Leu	Ala	Tyr	Ser	Ala	Thr	Thr	Ala	Arg	Gln	Glu
		20					25						30		
Glu	Val	Pro	Gln	Gln	Thr	Val	Ala	Pro	Gln	Gln	Gln	Arg	His	Ser	Phe
		35				40						45			
Lys	Gly	Glu	Glu	Cys	Pro	Ala	Gly	Ser	His	Arg	Ser	Glu	His	Thr	Gly
	50				55						60				
Ala	Cys	Asn	Pro	Cys	Thr	Glu	Gly	Val	Asp	Tyr	Thr	Asn	Ala	Ser	Asn
65				70					75					80	
Asn	Glu	Pro	Ser	Cys	Phe	Pro	Cys	Thr	Val	Cys	Lys	Ser	Asp	Gln	Lys
			85					90						95	
His	Lys	Ser	Ser	Cys	Thr	Met	Thr	Arg	Asp	Thr	Val	Cys	Gln	Cys	Lys
		100					105					110			
Glu	Gly	Thr	Phe	Arg	Asn	Glu	Asn	Ser	Pro	Glu	Met	Cys	Arg	Lys	Cys
	115					120						125			
Ser	Arg	Cys	Pro	Ser	Gly	Glu	Val	Gln	Val	Ser	Asn	Cys	Thr	Ser	Trp
130					135						140				

53

Asp Asp Ile Gln Cys Val Glu Glu Phe Gly Ala Asn Ala Thr Val Glu
 145 150 155 160
 Thr Pro Ala Ala Glu Glu Thr Met Asn Thr Ser Pro Gly Thr Pro Ala
 165 170 175
 Pro Ala Ala Glu Glu Thr Met Asn Thr Ser Pro Gly Thr Pro Ala Pro
 180 185 190
 Ala Ala Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Pro Ala
 195 200 205
 Ala Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala
 210 215 220
 Glu Glu Thr Met Thr Thr Ser Pro Gly Thr Pro Ala Ser Ser His Tyr
 225 230 235 240
 Leu Ser Cys Thr Ile Val Gly Ile Ile Val Leu Ile Val Leu Leu Ile
 245 250 255
 Val Phe Val

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 455 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
 1 5 10 15
 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
 20 25 30
 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
 35 40 45
 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
 50 55 60
 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
 65 70 75 80
 Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
 85 90 95
 Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
 100 105 110
 Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
 115 120 125
 Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
 130 135 140

54

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
 145 150 155 160
 Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
 165 170 175
 Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
 180 185 190
 Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
 195 200 205
 Gly Thr Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu
 210 215 220
 Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys
 225 230 235 240
 Ser Lys Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu
 245 250 255
 Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
 260 265 270
 Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val
 275 280 285
 Pro Ser Ser Thr Phe Thr Ser Ser Ser Thr Tyr Thr Pro Gly Asp Cys
 290 295 300
 Pro Asn Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly
 305 310 315 320
 Ala Asp Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn
 325 330 335
 Pro Leu Gln Lys Trp Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp
 340 345 350
 Thr Asp Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro
 355 360 365
 Leu Arg Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu
 370 375 380
 Ile Asp Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln
 385 390 395 400
 Tyr Ser Met Leu Ala Thr Trp Arg Arg Arg Thr Pro Arg Arg Glu Ala
 405 410 415
 Thr Leu Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly
 420 425 430
 Cys Leu Glu Asp Ile Glu Glu Ala Leu Cys Gly Pro Ala Ala Leu Pro
 435 440 445
 Pro Ala Pro Ser Leu Leu Arg
 450 455

55

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 461 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
1 5 10 15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
20 25 30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
35 40 45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
50 55 60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65 70 75 80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
85 90 95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
100 105 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
115 120 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
130 135 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145 150 155 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
165 170 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
180 185 190

Asn Ala Ser Arg Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
195 200 205

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
210 215 220

Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225 230 235 240

Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly
245 250 255

Asp Phe Ala Leu Pro Val Gly Leu Ile Val Gly Val Thr Ala Leu Gly

56

260	265	270
Leu Leu Ile Ile Gly Val Val	Asn Cys Val Ile Met Thr Gln Val Lys	
275	280	285
Lys Lys Pro Leu Cys Leu Gln Arg Glu Ala Lys Val Pro His Leu Pro		
290	295	300
Ala Asp Lys Ala Arg Gly Thr Gln Gly Pro Glu Gln Gln His Leu Leu		
305	310	315
Ile Thr Ala Pro Ser Ser Ser Ser Ser Ser Leu Glu Ser Ser Ala Ser		
325	330	335
Ala Leu Asp Arg Arg Ala Pro Thr Arg Asn Gln Pro Gln Ala Pro Gly		
340	345	350
Val Glu Ala Ser Gly Ala Gly Glu Ala Arg Ala Ser Thr Gly Ser Ser		
355	360	365
Asp Ser Ser Pro Gly Gly His Gly Thr Gln Val Asn Val Thr Cys Ile		
370	375	380
Val Asn Val Cys Ser Ser Ser Asp His Ser Ser Gln Cys Ser Ser Gln		
385	390	395
Ala Ser Ser Thr Met Gly Asp Thr Asp Ser Ser Pro Ser Glu Ser Pro		
405	410	415
Lys Asp Glu Gln Val Pro Phe Ser Lys Glu Glu Cys Ala Phe Arg Ser		
420	425	430
Gln Leu Glu Thr Pro Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys Pro		
435	440	445
Leu Pro Leu Gly Val Pro Asp Ala Gly Met Lys Pro Ser		
450	455	460

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 427 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gly Ala Gly Ala Thr Gly Arg Ala Met Asp Gly Pro Arg Leu Leu		
1	5	10
Leu Leu Leu Leu Leu Gly Val Ser Leu Gly Gly Ala Lys Glu Ala Cys		
20	25	30
Pro Thr Gly Leu Tyr Thr His Ser Gly Glu Cys Cys Lys Ala Cys Asn		
35	40	45
Leu Gly Glu Gly Val Ala Gln Pro Cys Gly Ala Asn Gln Thr Val Cys		

57

50 55 60
 Glu Pro Cys Leu Asp Ser Val Thr Phe Ser Asp Val Val Ser Ala Thr
 65 70 75 80
 Glu Pro Cys Lys Pro Cys Thr Glu Cys Val Gly Leu Gln Ser Met Ser
 85 90 95
 Ala Pro Cys Val Glu Ala Asp Asp Ala Val Cys Arg Cys Ala Tyr Gly
 100 105 110
 Tyr Tyr Gln Asp Glu Thr Thr Gly Arg Cys Glu Ala Cys Arg Val Cys
 115 120 125
 Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys Gln Asn Thr
 130 135 140
 Val Cys Glu Glu Cys Pro Asp Gly Thr Tyr Ser Asp Glu Ala Asn His
 145 150 155 160
 Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr Glu Arg Gln
 165 170 175
 Leu Arg Glu Cys Thr Arg Trp Ala Asp Ala Glu Cys Glu Glu Ile Pro
 180 185 190
 Gly Arg Trp Ile Thr Arg Ser Thr Pro Pro Glu Gly Ser Asp Ser Thr
 195 200 205
 Ala Pro Ser Thr Gln Glu Pro Glu Ala Pro Pro Glu Gln Asp Leu Ile
 210 215 220
 Ala Ser Thr Val Ala Gly Val Val Thr Thr Val Met Gly Ser Ser Gln
 225 230 235 240
 Pro Val Val Thr Arg Gly Thr Thr Asp Asn Leu Ile Pro Val Tyr Cys
 245 250 255
 Ser Ile Leu Ala Ala Val Val Val Gly Leu Val Ala Tyr Ile Ala Phe
 260 265 270
 Lys Arg Trp Asn Ser Cys Lys Gln Asn Lys Gln Gly Ala Asn Ser Arg
 275 280 285
 Pro Val Asn Gln Thr Pro Pro Glu Gly Glu Lys Leu His Ser Asp
 290 295 300
 Ser Gly Ile Ser Val Asp Ser Gln Ser Leu His Asp Gln Gln Pro His
 305 310 315 320
 Thr Gln Thr Ala Ser Gly Gln Ala Leu Lys Gly Asp Gly Gly Leu Tyr
 325 330 335
 Ser Ser Leu Pro Pro Ala Lys Arg Glu Glu Val Glu Lys Leu Leu Asn
 340 345 350
 Gly Ser Ala Gly Asp Thr Trp Arg His Leu Ala Gly Glu Leu Gly Tyr
 355 360 365
 Gln Pro Glu His Ile Asp Ser Phe Thr His Glu Ala Cys Pro Val Arg
 370 375 380
 Ala Leu Leu Ala Ser Trp Ala Thr Gln Asp Ser Ala Thr Leu Asp Ala

58

385 390 395 400

Leu Leu Ala Ala Leu Arg Arg Ile Gln Arg Ala Asp Leu Val Glu Ser
 405 410 415

Leu Cys Ser Glu Ser Thr Ala Thr Ser Pro Val
 420 425

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 415 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met 1	Arg	Leu	Pro	Arg 5	Ala	Ser	Ser	Pro	Cys 10	Gly	Leu	Ala	Trp	Gly 15	Pro
Leu	Leu	Leu	Gly 20	Leu	Ser	Gly	Leu	Leu 25	Val	Ala	Ser	Gln	Pro 30	Gln	Leu
Val	Pro	Pro 35	Tyr	Arg	Ile	Glu	Asn 40	Gln	Thr	Cys	Trp	Asp 45	Gln	Asp	Lys
Glu	Tyr 50	Tyr	Glu	Pro	Met	His 55	Asp	Val	Cys	Cys	Ser 60	Arg	Cys	Pro	Pro
Gly 65	Glu	Phe	Val	Phe	Ala 70	Val	Cys	Ser	Arg	Ser 75	Gln	Asp	Thr	Val	Cys 80
Lys	Thr	Cys	Pro	His 85	Asn	Ser	Tyr	Asn	Glu 90	His	Trp	Asn	His	Leu 95	Ser
Thr	Cys	Gln	Leu 100	Cys	Arg	Pro	Cys	Asp 105	Ile	Val	Leu	Gly	Phe 110	Glu	Glu
Val	Ala	Pro 115	Cys	Thr	Ser	Asp	Arg 120	Lys	Ala	Glu	Cys	Arg 125	Cys	Gln	Pro
Gly 130	Met	Ser	Cys	Val	Tyr	Leu 135	Asp	Asn	Glu	Cys	Val 140	His	Cys	Glu	Glu
Glu 145	Arg	Leu	Val	Leu	Cys 150	Gln	Pro	Gly	Thr	Glu 155	Ala	Glu	Val	Thr	Asp 160
Glu	Ile	Met	Asp	Thr 165	Asp	Val	Asn	Cys	Val 170	Pro	Cys	Lys	Pro	Gly 175	His
Phe	Gln	Asn	Thr 180	Ser	Ser	Pro	Arg	Ala 185	Arg	Cys	Gln	Pro	His 190	Thr	Arg
Cys	Glu	Ile 195	Gln	Gly	Leu	Val	Glu 200	Ala	Ala	Pro	Gly	Thr 205	Ser	Tyr	Ser
Asp	Thr	Ile	Cys	Lys	Asn	Pro	Pro	Glu	Pro	Gly	Ala	Met	Leu	Leu	Leu

210	215	220
Ala Ile Leu Leu Ser Leu Val Leu Phe Leu Leu Phe Thr Thr Val Leu		
225	230	235 240
Ala Cys Ala Trp Met Arg His Pro Ser Leu Cys Arg Lys Leu Gly Thr		
245	250	255
Leu Leu Lys Arg His Pro Glu Gly Glu Glu Ser Pro Pro Cys Pro Ala		
260	265	270
Pro Arg Ala Asp Pro His Phe Pro Asp Leu Ala Glu Pro Leu Leu Pro		
275	280	285
Met Ser Gly Asp Leu Ser Pro Ser Pro Ala Gly Pro Pro Thr Ala Pro		
290	295	300
Ser Leu Glu Glu Val Val Leu Gln Gln Gln Ser Pro Leu Val Gln Ala		
305	310	315 320
Arg Glu Leu Glu Ala Glu Pro Gly Glu His Gly Gln Val Ala His Gly		
325	330	335
Ala Asn Gly Ile His Val Thr Gly Gly Ser Val Thr Val Thr Gly Asn		
340	345	350
Ile Tyr Ile Tyr Asn Gly Pro Val Leu Gly Gly Thr Arg Gly Pro Gly		
355	360	365
Asp Pro Pro Ala Pro Pro Glu Pro Pro Tyr Pro Thr Pro Glu Glu Gly		
370	375	380
Ala Pro Gly Pro Ser Glu Leu Ser Thr Pro Tyr Gln Glu Asp Gly Lys		
385	390	395 400
Ala Trp His Leu Ala Glu Thr Glu Thr Leu Gly Cys Gln Asp Leu		
405	410	415

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 335 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala		
1	5	10 15
Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser		
20	25	30
Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn		
35	40	45
Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro		
50	55	60

60


```

Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65                               70                               75                               80

Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
                               85                               90                               95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
                               100                              105                              110

Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
                               115                              120                              125

Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp
                               130                              135                              140

Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
145                               150                              155                              160

Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
                               165                              170                              175

Leu Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
                               180                              185                              190

Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
                               195                              200                              205

Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu
210                               215                              220

Ser Asp Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met
225                               230                              235                              240

Thr Leu Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu
                               245                              250                              255

Ala Lys Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu
                               260                              265                              270

Gln Lys Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys
275                               280                              285

Glu Ala Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys
290                               295                              300

Thr Leu Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser
305                               310                              315                              320

Asp Ser Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val
                               325                              330                              335

```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 260 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

61

```

Met Ala Arg Pro His Pro Trp Trp Leu Cys Val Leu Gly Thr Leu Val
1           5           10           15

Gly Leu Ser Ala Thr Pro Ala Pro Lys Ser Cys Pro Glu Arg His Tyr
          20           25           30

Trp Ala Gln Gly Lys Leu Cys Cys Gln Met Cys Glu Pro Gly Thr Phe
          35           40           45

Leu Val Lys Asp Cys Asp Gln His Arg Lys Ala Ala Gln Cys Asp Pro
          50           55           60

Cys Ile Pro Gly Val Ser Phe Ser Pro Asp His His Thr Arg Pro His
          65           70           75           80

Cys Glu Ser Cys Arg His Cys Asn Ser Gly Leu Leu Val Arg Asn Cys
          85           90           95

Thr Ile Thr Ala Asn Ala Glu Cys Ala Cys Arg Asn Gly Trp Gln Cys
          100          105          110

Arg Asp Lys Glu Cys Thr Glu Cys Asp Pro Leu Pro Asn Pro Ser Leu
          115          120          125

Thr Ala Arg Ser Ser Gln Ala Leu Ser Pro His Pro Gln Pro Thr His
          130          135          140

Leu Pro Tyr Val Ser Glu Met Leu Glu Ala Arg Thr Ala Gly His Met
          145          150          155          160

Gln Thr Leu Ala Asp Phe Arg Gln Leu Pro Ala Arg Thr Leu Ser Thr
          165          170          175

His Trp Pro Pro Gln Arg Ser Leu Cys Ser Ser Asp Phe Ile Arg Ile
          180          185          190

Leu Val Ile Phe Ser Gly Met Phe Leu Val Phe Thr Leu Ala Gly Ala
          195          200          205

Leu Phe Leu His Gln Arg Arg Lys Tyr Arg Ser Asn Lys Gly Glu Ser
          210          215          220

Pro Val Glu Pro Ala Glu Pro Cys Arg Tyr Ser Cys Pro Arg Glu Glu
          225          230          235          240

Glu Gly Ser Thr Ile Pro Ile Gln Glu Asp Tyr Arg Lys Pro Glu Pro
          245          250          255

Ala Cys Ser Pro
          260

```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 595 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

Met Arg Val Leu Leu Ala Ala Leu Gly Leu Leu Phe Leu Gly Ala Leu
1           5           10           15

Arg Ala Phe Pro Gln Asp Arg Pro Phe Glu Asp Thr Cys His Gly Asn
20           25           30

Pro Ser His Tyr Tyr Asp Lys Ala Val Arg Arg Cys Cys Tyr Arg Cys
35           40           45

Pro Met Gly Leu Phe Pro Thr Gln Gln Cys Pro Gln Arg Pro Thr Asp
50           55           60

Cys Arg Lys Gln Cys Glu Pro Asp Tyr Tyr Leu Asp Glu Ala Asp Arg
65           70           75           80

Cys Thr Ala Cys Val Thr Cys Ser Arg Asp Asp Leu Val Glu Lys Thr
85           90           95

Pro Cys Ala Trp Asn Ser Ser Arg Val Cys Glu Cys Arg Pro Gly Met
100          105          110

Phe Cys Ser Thr Ser Ala Val Asn Ser Cys Ala Arg Cys Phe Phe His
115          120          125

Ser Val Cys Pro Ala Gly Met Ile Val Lys Phe Pro Gly Thr Ala Gln
130          135          140

Lys Asn Thr Val Cys Glu Pro Ala Ser Pro Gly Val Ser Pro Ala Cys
145          150          155          160

Ala Ser Pro Glu Asn Cys Lys Glu Pro Ser Ser Gly Thr Ile Pro Gln
165          170          175

Ala Lys Pro Thr Pro Val Ser Pro Ala Thr Ser Ser Ala Ser Thr Met
180          185          190

Pro Val Arg Gly Gly Thr Arg Leu Ala Gln Glu Ala Ala Ser Lys Leu
195          200          205

Thr Arg Ala Pro Asp Ser Pro Ser Ser Val Gly Arg Pro Ser Ser Asp
210          215          220

Pro Gly Leu Ser Pro Thr Gln Pro Cys Pro Glu Gly Ser Gly Asp Cys
225          230          235          240

Arg Lys Gln Cys Glu Pro Asp Tyr Tyr Leu Asp Glu Ala Gly Arg Cys
245          250          255

Thr Ala Cys Val Ser Cys Ser Arg Asp Asp Leu Val Glu Lys Thr Pro
260          265          270

Cys Ala Trp Asn Ser Ser Arg Thr Cys Glu Cys Arg Pro Gly Met Ile
275          280          285

Cys Ala Thr Ser Ala Thr Asn Ser Cys Ala Arg Cys Val Pro Tyr Pro
290          295          300

Ile Cys Ala Ala Glu Thr Val Thr Lys Pro Gln Asp Met Ala Glu Lys
305          310          315          320

Asp Thr Thr Phe Glu Ala Pro Pro Leu Gly Thr Gln Pro Asp Cys Asn

```

63

325	330	335
Pro Thr Pro Glu Asn Gly Glu Ala Pro Ala Ser Thr Ser Pro Thr Gln		
340	345	350
Ser Leu Leu Val Asp Ser Gln Ala Ser Lys Thr Leu Pro Ile Pro Thr		
355	360	365
Ser Ala Pro Val Ala Leu Ser Ser Thr Gly Lys Pro Val Leu Asp Ala		
370	375	380
Gly Pro Val Leu Phe Trp Val Ile Leu Val Leu Val Val Val Gly		
385	390	395
Ser Ser Ala Phe Leu Leu Cys His Arg Arg Ala Cys Arg Lys Arg Ile		
405	410	415
Arg Gln Lys Leu His Leu Cys Tyr Pro Val Gln Thr Ser Gln Pro Lys		
420	425	430
Leu Glu Leu Val Asp Ser Arg Pro Arg Arg Ser Ser Thr Gln Leu Arg		
435	440	445
Ser Gly Ala Ser Val Thr Glu Pro Val Ala Glu Glu Arg Gly Leu Met		
450	455	460
Ser Gln Pro Leu Met Glu Thr Cys His Ser Val Gly Ala Ala Tyr Leu		
465	470	475
Glu Ser Leu Pro Leu Gln Asp Ala Ser Pro Ala Gly Gly Pro Ser Ser		
485	490	495
Pro Arg Asp Leu Pro Glu Pro Arg Val Ser Thr Glu His Thr Asn Asn		
500	505	510
Lys Ile Glu Lys Ile Tyr Ile Met Lys Ala Asp Thr Val Ile Val Gly		
515	520	525
Thr Val Lys Ala Glu Leu Pro Glu Gly Arg Gly Leu Ala Gly Pro Ala		
530	535	540
Glu Pro Glu Leu Glu Glu Glu Leu Glu Ala Asp His Thr Pro His Tyr		
545	550	555
Pro Glu Gln Glu Thr Glu Pro Pro Leu Gly Ser Cys Ser Asp Val Met		
565	570	575
Leu Ser Val Glu Glu Glu Gly Lys Glu Asp Pro Leu Pro Thr Ala Ala		
580	585	590
Ser Gly Lys		
595		

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 277 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

44

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
1           5           10           15

Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
          20           25           30

Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
          35           40           45

Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
          50           55           60

Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
          65           70           75           80

Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
          85           90           95

Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
          100          105          110

Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly
          115          120          125

Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu
          130          135          140

Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
          145          150          155          160

Cys His Pro Trp Thr Ser Cys Glu Thr Lys Asp Leu Val Val Gln Gln
          165          170          175

Ala Gly Thr Asn Lys Thr Asp Val Val Cys Gly Pro Gln Asp Arg Leu
          180          185          190

Arg Ala Leu Val Val Ile Pro Ile Ile Phe Gly Ile Leu Phe Ala Ile
          195          200          205

Leu Leu Val Leu Val Phe Ile Lys Lys Val Ala Lys Lys Pro Thr Asn
          210          215          220

Lys Ala Pro His Pro Lys Gln Glu Pro Gln Glu Ile Asn Phe Pro Asp
          225          230          235          240

Asp Leu Pro Gly Ser Asn Thr Ala Ala Pro Val Gln Glu Thr Leu His
          245          250          255

Gly Cys Gln Pro Val Thr Gln Glu Asp Gly Lys Glu Ser Arg Ile Ser
          260          265          270

Val Gln Glu Arg Gln
          275

```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 255 amino acids

65

- (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val Leu
1           5           10           15

Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro
          20           25           30

Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys
          35           40           45

Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile
50           55           60

Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser
65           70           75           80

Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly
          85           90           95

Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu
          100          105          110

Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln
          115          120          125

Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys
          130          135          140

Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro
          145          150          155          160

Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala
          165          170          175

Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu
          180          185          190

Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu
          195          200          205

Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
          210          215          220

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
          225          230          235          240

Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
          245          250          255

```

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 277 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single

66

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Cys Val Gly Ala Arg Arg Leu Gly Arg Gly Pro Cys Ala Ala Leu
1           5           10           15

Leu Leu Leu Gly Leu Gly Leu Ser Thr Val Thr Gly Leu His Cys Val
20           25           30

Gly Asp Thr Tyr Pro Ser Asn Asp Arg Cys Cys His Glu Cys Arg Pro
35           40           45

Gly Asn Gly Met Val Ser Arg Cys Ser Arg Ser Gln Asn Thr Val Cys
50           55           60

Arg Pro Cys Gly Pro Gly Phe Tyr Asn Asp Val Val Ser Ser Lys Pro
65           70           75           80

Cys Lys Pro Cys Thr Trp Cys Asn Leu Arg Ser Gly Ser Glu Arg Lys
85           90           95

Gln Leu Cys Thr Ala Thr Gln Asp Thr Val Cys Arg Cys Arg Ala Gly
100          105          110

Thr Gln Pro Leu Asp Ser Tyr Lys Pro Gly Val Asp Cys Ala Pro Cys
115          120          125

Pro Pro Gly His Phe Ser Pro Gly Asp Asn Gln Ala Cys Lys Pro Trp
130          135          140

Thr Asn Cys Thr Leu Ala Gly Lys His Thr Leu Gln Pro Ala Ser Asn
145          150          155          160

Ser Ser Asp Ala Ile Cys Glu Asp Arg Asp Pro Pro Ala Thr Gln Pro
165          170          175

Gln Glu Thr Gln Gly Pro Pro Ala Arg Pro Ile Thr Val Gln Pro Thr
180          185          190

Glu Ala Trp Pro Arg Thr Ser Gln Gly Pro Ser Thr Arg Pro Val Glu
195          200          205

Val Pro Gly Gly Arg Ala Val Ala Ala Ile Leu Gly Leu Gly Leu Val
210          215          220

Leu Gly Leu Leu Gly Pro Leu Ala Ile Leu Leu Ala Leu Tyr Leu Leu
225          230          235          240

Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly
245          250          255

Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
260          265          270

Thr Leu Ala Lys Ile
275

```

67

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 349 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```

Met Lys Ser Val Leu Tyr Leu Tyr Ile Leu Phe Leu Ser Cys Ile Ile
1           5           10           15

Ile Asn Gly Arg Asp Ala Ala Pro Tyr Thr Pro Pro Asn Gly Lys Cys
          20           25           30

Lys Asp Thr Glu Tyr Lys Arg His Asn Leu Cys Cys Leu Ser Cys Pro
          35           40           45

Pro Gly Thr Tyr Ala Ser Arg Leu Cys Asp Ser Lys Thr Asn Thr Gln
          50           55           60

Cys Thr Pro Cys Gly Ser Gly Thr Phe Thr Ser Arg Asn Asn His Leu
65           70           75           80

Pro Ala Cys Leu Ser Cys Asn Gly Arg Cys Asn Ser Asn Gln Val Glu
          85           90           95

Thr Arg Ser Cys Asn Thr Thr His Asn Arg Ile Cys Glu Cys Ser Pro
          100          105          110

Gly Tyr Tyr Cys Leu Leu Lys Gly Ser Ser Gly Cys Lys Ala Cys Val
          115          120          125

Ser Gln Thr Lys Cys Gly Ile Gly Tyr Gly Val Ser Gly His Thr Ser
          130          135          140

Val Gly Asp Val Ile Cys Ser Pro Cys Gly Phe Gly Thr Tyr Ser His
          145          150          155          160

Thr Val Ser Ser Ala Asp Lys Cys Glu Pro Val Pro Asn Asn Thr Phe
          165          170          175

Asn Tyr Ile Asp Val Glu Ile Thr Leu Tyr Pro Val Asn Asp Thr Ser
          180          185          190

Cys Thr Arg Thr Thr Thr Thr Gly Leu Ser Glu Ser Ile Leu Thr Ser
          195          200          205

Glu Leu Thr Ile Thr Met Asn His Thr Asp Cys Asn Pro Val Phe Arg
          210          215          220

Glu Glu Tyr Phe Ser Val Leu Asn Lys Val Ala Thr Ser Gly Phe Phe
          225          230          235          240

Thr Gly Glu Asn Arg Tyr Gln Asn Ile Ser Lys Val Cys Thr Leu Asn
          245          250          255

Phe Glu Ile Lys Cys Asn Asn Lys Gly Ser Ser Phe Lys Gln Leu Thr
          260          265          270

```

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Lys Ala Lys Asn Asp Asp Gly Met Met Ser His Ser Glu Thr Val Thr
 275 280 285
 Leu Ala Gly Asp Cys Leu Ser Ser Val Asp Ile Tyr Ile Leu Tyr Ser
 290 295 300
 Asn Thr Asn Ala Gln Asp Tyr Glu Thr Asp Thr Ile Ser Tyr Arg Val
 305 310 315 320
 Gly Asn Val Leu Asp Asp Asp Ser His Met Pro Gly Ser Cys Asn Ile
 325 330 335
 His Lys Pro Ile Thr Asn Ser Lys Pro Thr Arg Phe Leu
 340 345

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 355 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Lys Ser Tyr Ile Leu Leu Leu Leu Leu Ser Cys Ile Ile Ile Ile
 1 5 10 15
 Asn Ser Asp Ile Thr Pro His Glu Pro Ser Asn Gly Lys Cys Lys Asp
 20 25 30
 Asn Glu Tyr Lys Arg His His Leu Cys Cys Leu Ser Cys Pro Pro Gly
 35 40 45
 Thr Tyr Ala Ser Arg Leu Cys Asp Ser Lys Thr Asn Thr Asn Thr Gln
 50 55 60
 Cys Thr Pro Cys Ala Ser Asp Thr Phe Thr Ser Arg Asn Asn His Leu
 65 70 75 80
 Pro Ala Cys Leu Ser Cys Asn Gly Arg Cys Asp Ser Asn Gln Val Glu
 85 90 95
 Thr Arg Ser Cys Asn Thr Thr His Asn Arg Ile Cys Asp Cys Ala Pro
 100 105 110
 Gly Tyr Tyr Cys Phe Leu Lys Gly Ser Ser Gly Cys Lys Ala Cys Val
 115 120 125
 Ser Gln Thr Lys Cys Gly Ile Gly Tyr Gly Val Ser Gly His Thr Pro
 130 135 140
 Thr Gly Asp Val Val Cys Ser Pro Cys Gly Leu Gly Thr Tyr Ser His
 145 150 155 160
 Thr Val Ser Ser Val Asp Lys Cys Glu Pro Val Pro Ser Asn Thr Phe
 165 170 175
 Asn Tyr Ile Asp Val Glu Ile Asn Leu Tyr Pro Val Asn Asp Thr Ser

69

	180						185						190					
Cys Thr Arg Thr Thr Thr Thr Gly Leu Ser Glu Ser Ile Ser Thr Ser																		
195							200						205					
Glu Leu Thr Ile Thr Met Asn His Lys Asp Cys Asp Pro Val Phe Arg																		
210							215						220					
Asn Gly Tyr Phe Ser Val Leu Asn Glu Val Ala Thr Ser Gly Phe Phe																		
225							230						235					
Thr Gly Gln Asn Arg Tyr Gln Asn Ile Ser Lys Val Cys Thr Leu Asn																		
	245						250						255					
Phe Glu Ile Lys Cys Asn Asn Lys Asp Ser Tyr Ser Ser Ser Lys Gln																		
	260						265						270					
Leu Thr Lys Thr Lys Asn Asp Asp Asp Ser Ile Met Pro His Ser Glu																		
	275						280						285					
Ser Val Thr Leu Val Gly Asp Cys Leu Ser Ser Val Asp Ile Tyr Ile																		
	290						295						300					
Leu Tyr Ser Asn Thr Asn Thr Gln Asp Tyr Glu Thr Asp Thr Ile Ser																		
305	310						315						320					
Tyr His Val Gly Asn Val Leu Asp Val Asp Ser His Met Pro Gly Arg																		
	325						330						335					
Cys Asp Thr His Lys Leu Ile Thr Asn Ser Asn Ser Gln Tyr Pro Thr																		
	340						345						350					
His Phe Leu																		
	355																	

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 506 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTTCGGCA	NAGCCTCTCC	ACGCGCAGAA	CTCAGCCAAC	GATTTCCTGAT	AGATTTTGTG	60
GAGTTTGACC	AGAGATGCAA	GGGGTGAAGG	AGCGCTTCCT	ACCGTTAGGA	ACTCTGGGGA	120
CAGNNCGCCC	CGGCCGCCTG	ATGGCCGAGG	CAGGGTGCGA	CCCAGGACCC	AGGACGGCGT	180
CGGGAACCAT	ACCATGGCCC	GGATCCCCAA	GACCCATAAG	TTCGTGGTCG	TCATCGTCGC	240
GGTCTGTCTG	CCAGTCCTAG	CTTACTCTGC	CACCACTGCC	CGGCAGAGGA	AGTTNCCAG	300
CAGNCANTGG	NCCCACAGCA	ACAGNGGCAC	AGTTTCAAGG	GGGNAGGAGT	TTTCCANCAA	360
GTTTTTATAG	TTCAGAACNT	ATTGGNGCTN	TNAACCCCTT	CACAAGGGTT	TGGNTTAAAC	420

70

CAANGTTTCC AANATGNACT TTTTNGTTCC CTGTTANATT TTTTAATTAG TTNAANTTAA 480
ATTTNTNAAC CTTNCCNGGG NAAATT 506

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 325 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGCAGAGGTG TCTCCAGCCT GGCTCTATCT TCCTCCTTGT NATCGTCCCA TCCCCACATC 60
CCGTGCACCC CCCAGGACCC TGGTCTCATC AGTCCCTCTC CTGGAGCTGG GGGTCCACAC 120
ATCTCCCAGC CAAGTCCAAG AGGGCAGGGC CAGTTCCTCC CATCTTCAGG CCCAGCCAGG 180
CAGGGGGCAG TCGGCTCCTC AACTGGGTGA CAAGGGTGAG GATGAGAACT GGTACCGGGG 240
ATTTATTTCAG CCTTGGTCAG AGCAGAACAC AGATTTTTC GTGTGTTGGT TTTTACTCTN 300
NTTCCCCTTC TTNATNCCCC TTTCN 325

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 340 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGCAGAGGCC CCAGCTGCTG AAGAGACAAT AATCACCAGC CCGGGGACTC CTGNNTCTNC 60
TNATTACCTC TNATGCACCA TCGTAGGGAT CATAGTTCTA ATTGTGCCTT CTAATGTTT 120
TTGTTTGAAA AGANTTCACT GTGGAAGAAA TTCCTTCCTT ACCTGTAAGT TNCAGGTAGG 180
NGCCTGGCTG AGGGCGGGGG GCGCTGGTAC ACTCTCTGAC CCTGCCTCCC TCTGNCTGTT 240
TTCCCACAGA CAGAAACGCC TGCCCCTGNC CCCAAGTTCC TNGTGTTTTT CAGCCTGGCT 300
CTATCTTNNC TCCTTGTGAA TCGTTCCCAT CCCCACANGC 340

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

71

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
CCAGGGTCTC CTNCCCCACC TGCTGAAGAG ACANTGACCA CCAGCCCGGG GACTCCTGCC      60
TCTTCCTCAT TACCTCTNAT GNANCATCGT AGGGATCATA GTTCTAATTG TGCCTTCTGA      120
ATTGTGCTTT GTTTGGAAAG ACTTCACTGT GGAAGAAAT TCCTTCCTTA CCTGAAGTTG      180
CAGGTAGGCC CTGGGTNAGG GCGNGGGGCG CTGGACANTN TCTGGNCCTG GCTGCCCGCT      240
G                                                                              241
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGCGGATCCA CCACTGCCCC GCAGGAG

27

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCGTCTAGAC TAGTAATGAG AAGAGGCAGG
30

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

72

CGCTCTAGAC CGCCATCATG GCCCGGATCC CCAAG

37

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCGTCTAGAC TAGTAATGAG AAGAGGCAGG

30

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGCGAATTCC GCCATCATGG CCCGGATCCC CAAG

34

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCGTCTAGAG TAATGAGAAG AGGCAGG

27

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

73

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGCTCTAGAC CGCCATCATG GCCCGGATCC CCAAG

35

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GCGTCTAGAC TAGTAATGAG AAGAGGCAGG

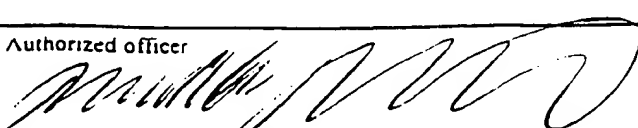
33

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>4</u> , line <u>5</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet: <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 12301 Parklawn Drive Rockville, Maryland 20852 United States of America	
Date of deposit November 20, 1996	Accession Number 97798
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet: <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="border: 1px solid black; padding: 5px;">For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer </div>	<div style="border: 1px solid black; padding: 5px;">For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer</div>

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a TRID polypeptide having the complete amino acid sequence in SEQ ID NO:2, or the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798;

(b) a nucleotide sequence encoding a mature TRID polypeptide having the amino acid sequence at positions 1-233 in SEQ ID NO:2, or the mature polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97798;

(c) a nucleotide sequence encoding the soluble extracellular domain of a TRID polypeptide having the amino acid sequence at positions 1-214 of SEQ ID NO:2, or the extracellular domain encoded by the cDNA clone contained in ATCC Deposit No. 97798; and

(d) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b) or (c) above.

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has a complete nucleotide of SEQ ID NO:1.

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has a nucleotide sequence which encodes a TRID polypeptide having the complete amino acid of SEQ ID NO:2.

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has a nucleotide sequence encoding the mature form of a TRID polypeptide having an amino acid sequence from about 1 to about 233 in SEQ ID NO:2.

5. The nucleic acid molecule of claim 1 wherein said polynucleotide has a nucleotide sequence encoding the soluble extracellular domain of a TRID polypeptide having the amino acid sequence from about 1 to about 214 in SEQ ID NO:2.

6. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues m-233 of SEQ ID NO:2, where m is an integer in the range of -1 to 27;

(b) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues 1-x of SEQ ID NO:2, where x is an integer in the range of 123-233;

(c) a nucleotide sequence encoding a polypeptide having the amino acid sequence consisting of residues m-x of SEQ ID NO:2, m and x are defined in (a) and (b) above.

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7. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide consisting of a portion of a complete TRID amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798 wherein said portion excludes from 1 to about 52 amino acids from the amino terminus of said complete amino acid sequence;

(b) a nucleotide sequence encoding a polypeptide consisting of a portion of a complete TRID amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 97798 wherein said portion excludes from 1 to about 110 amino acids from the carboxy terminus of said complete amino acid sequence; and

(c) a nucleotide sequence encoding a polypeptide consisting of a portion of a complete TRID amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798, wherein said portion includes a combination of any of the amino terminal and carboxy terminal deletions for the respective clones in (a) and (b), above.

8. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 97798.

9. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding a TRID polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798.

10. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding a mature TRID polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97798.

11. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), or (d) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

12. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a TRID polypeptide having an amino acid sequence in (a), (b), (c) or (d) of claim 1.

13. The isolated nucleic acid molecule of claim 12, which encodes an epitope-bearing portion of a TRID polypeptide comprising amino acid residues selected from the group consisting of: from about Gln-42 to about Glu-52 in Figure 1, from about His-58 to about Cys-66 in Figure 1, from about Pro-68 to about Thr-76 in Figure 1, from about Ser-79 to about Cys-85 in Figure 1, from about Cys-91 to about Thr-102 in Figure 1, from about Gln-110 to about Pro-122 in Figure 1, from about Arg-126 to about Val-136 in Figure 1, and from about Thr-142 to about Glu-148 in Figure 1.

14. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

15. A recombinant vector produced by the method of claim 14.

16. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 15 into a host cell.

17. A recombinant host cell produced by the method of claim 16.

18. A recombinant method for producing a TRID polypeptide, comprising culturing the recombinant host cell of claim 17 under conditions such that said polypeptide is expressed and recovering said polypeptide.

19. An isolated TRID polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of a full-length TRID polypeptide having the complete amino acid sequence shown in SEQ ID NO:2, or as encoded by the cDNA clone contained in ATCC Deposit No. 97798;

(b) the amino acid sequence of a mature TRID polypeptide having the amino acid sequence at positions 1-233 in SEQ ID NO:2, or as encoded by the cDNA clone contained in ATCC Deposit No. 97798; or

(c) the amino acid sequence of a soluble extracellular domain of a TRID polypeptide having the amino acid sequence at positions 1 to 216 in SEQ ID NO:2, or as encoded by the cDNA clone contained in ATCC Deposit No. 97798.

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20. An isolated polypeptide comprising an epitope-bearing portion of the TNFR protein, wherein said portion is selected from the group consisting of a polypeptide comprising amino acid residues: from about Gln-42 to about Glu-52 in Figure 1, from about His-58 to about Cys-66 in Figure 1, from about Pro-68 to about Thr-76 in Figure 1, from about Ser-79 to about Cys-85 in Figure 1, from about Cys-91 to about Thr-102 in Figure 1, from about Gln-110 to about Pro-122 in Figure 1, from about Arg-126 to about Val-136 in Figure 1, and from about Thr-142 to about Glu-148 in Figure 1.

21. An isolated antibody that binds specifically to a TRID polypeptide of claim 19.

22. A method for treating a patient in need of TRID polypeptide activity comprising administering to said patient a therapeutically effective amount of the polypeptide of claim 19.

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CCTCTCCACGCGCACGAACTCAGCCAACGATTTCTGATAGATTTTTGGGAGTTTGACCAG
AGATGCAAGGGGTGAAGGAGCGCTTCCTACCGTTAGGGAACTCTGGGGACAGAGCGCCCC
GGCCGCCTGATGGCCGAGGCAGGGTGCGACCCAGGACCCAGGACGGCGTCGGGAACCATA
CCATGGCCCGGATCCCCAAGACCCTAAAGTTCGTCGTCGTCATCGTCGCGGTCCTGCTGC
M A R I P K T L K F V V V I V A V L L P
CAGTCCTAGCTTACTCTGCCACCACTGCCCAGGAGGAAGTTCCCCAGCAGACAGTGG
V L A Y S A T T A R Q E E V P Q Q T V A
CCCCACAGCAACAGAGGCACAGCTTCAAGGGGGAGGAGTGTCCAGCAGGATCTCATAGAT
P Q Q Q R H S F K G E E C P A G S H R S
CAGAACATACTGGAGCCTGTAACCCGTGCACAGAGGGTGTGGATTACACCAACGCTTCCA
E H T G A C N P C T E G V D Y T N A S N
ACAATGAACCTTCTTGCTTCCCATGTACAGTTTGTAATCAGATCAAAAACATAAAAGTT
N E P S C F P C T V C K S D Q K H K S S
CCTGCACCATGACCAGAGACACAGTGTGTCAGTGTAAAGAAGGCACCTTCCGGAATGAAA
C T M T R D T V C Q C K E G T F R N E N
ACTCCCCAGAGATGTGCCGGAAGTGTAGCAGGTGCCCTAGTGGGGAAGTCCAAGTCAGTA
S P E M C R K C S R C P S G E V Q V S N
ATTGTACGTCTGGGATGATATCCAGTGTGTTGAAGAATTTGGTGCCAATGCCACTGTGG
C T S W D D I Q C V E E F G A N A T V E
AAACCCCAGCTGCTGAAGAGACAATGAACACCAGCCCGGGGACTCCTGCCCCAGCTGCTG
T P A A E E T M N T S P G T P A P A A E
AAGAGACAATGAACACCAGCCCAGGGACTCCTGCCCCAGCTGCTGAAGAGACAATGACCA
E T M N T S P G T P A P A A E E T M T T
CCAGCCCGGGGACTCCTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCCGGGGACTC
S P G T P A P A A E E T M T T S P G T P
CTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCCGGGGACTCCTGCCTCTTCTCATT
A P A A E E T M T T S P G T P A S S H Y

FIG. 1

SUBSTITUTE SHEET (RULE 26)

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ACCTCTCATGCACCATCGTAGGGATCATAGTTCTAATTGTGCTTCTGATTGTGTTTGTTT
L S C T I V G I I V L I V L L I V F V *
GAAAGACTTCACTGTGGAAGAAATTCCTTCCTTACCTGAAAGGTTCAAGTAGGCGCTGGC
TGAGGGCGGGGGCGCTGGACACTCTCTGCCCTGCCTCCCTCTGCTGTGTTCCACAGAC
AGAAACGCCTGCCCCTGCCCCAAGTCCTGGTGTCTCCAGCCTGGCTCTATCTTCCTCCTT
GTGATCGTCCCATCCCCACATCCCGTGCACCCCCCAGGACCCTGGTCTCATCAGTCCCTC
TCCTGGAGCTGGGGTCCACACATCTCCCAGCCAAGTCCAAGAGGCAGGGCCAGTTCCTC
CCATCTTCAGGCCCAGCCAGGCAGGGGGCAGTCGGCTCCTCAACTGGGTGACAAGGGTGA
GGATGAGAAGTGGTCACGGGATTTATTCAGCCTTGGTCAGAGCAGAACACAGAGATTTTC
CGTGAAAAAAAA

FIG. 1B

1	M	-GL	S	T	V	P	D	L	L	V	L	E	L	L	V	G	I	Y	P	S	G	V	I	G	L	V	P	H	L	G	D	R	E	-	TNFR1					
1	M	A	P	V	A	W	A	A	L	L	E	L	W	A	A	H	A	L	P	A	Q	V	A	-	-	-	-	-	F	T	P	-	-	TNFR2						
1	M	G	A	G	A	T	G	R	A	M	D	-	-	G	P	R	L	L	L	L	L	G	V	S	L	G	A	K	E	-	A	C	P	-	-	NGFR				
1	M	-R	L	P	R	-	A	S	S	P	C	G	L	A	W	G	P	L	L	L	G	L	S	G	L	V	A	S	Q	P	Q	L	V	P	-	-	LTBR			
1	M	L	G	I	W	T	-	-	-	L	L	P	L	V	L	T	S	V	-	A	R	L	S	S	K	S	V	N	A	Q	V	T	D	I	N	S	K	G	L	FAS
1	M	-	-	-	-	A	R	P	H	P	-	-	W	L	C	V	L	G	T	L	V	G	L	S	-	A	T	P	A	P	K	S	C	P	-	-	CD27			
1	M	R	V	L	-	-	-	-	-	-	-	-	-	L	A	A	L	G	L	L	F	L	G	A	L	R	A	-	-	-	-	F	P	Q	-	-	CD30			
1	M	V	R	L	P	L	-	Q	-	-	C	V	L	-	W	G	C	L	L	-	-	-	-	-	T	A	V	H	P	E	-	-	P	P	-	-	CD40			
1	M	G	N	-	-	-	-	-	S	C	Y	N	I	V	A	T	L	L	L	V	L	N	-	-	-	-	-	-	-	-	-	F	E	R	-	-	4-1BB			
1	M	C	V	G	A	-	-	R	R	L	G	-	-	R	G	P	C	A	A	L	L	L	G	L	G	L	S	T	V	T	G	L	H	C	V	-	-	OX40		
1	M	K	S	V	-	L	Y	L	I	-	-	L	F	L	S	C	I	I	I	N	G	R	D	A	A	P	-	-	-	-	-	Y	T	P	-	-	VC22			
1	M	K	S	-	-	Y	I	L	L	-	-	L	L	L	S	C	I	I	I	N	S	D	I	T	P	-	-	-	-	-	H	E	P	-	-	CRM6				
1	M	-A	R	I	P	K	T	L	K	F	V	V	I	V	A	V	L	L	P	V	L	A	Y	S	A	T	T	-	-	-	-	-	-	A	R	Q	E	TNFR-like		

FIG. 2A

39	-	-	K	R	D	S	V	C	P	Q	G	K	Y	I	H	-	-	P	Q	N	N	S	I	C	C	T	K	C	H	K	G	T	Y	L	Y	N	D	C	P	G	TNFR1	
32	-	-	Y	-	A	P	E	P	G	S	T	C	R	L	R	E	Y	Y	D	Q	T	A	Q	M	C	C	S	K	C	S	P	G	Q	H	A	K	V	F	C	-	-	TNFR2
34	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	G	L	Y	T	H	S	G	E	-	C	C	K	A	C	N	L	G	E	G	V	A	Q	P	C	G	A	NGFR
36	-	-	Y	R	I	E	N	Q	T	C	W	D	Q	D	K	E	Y	Y	E	P	M	H	D	V	C	C	S	R	C	P	P	G	E	F	V	F	A	V	C	-	-	LTR
36	E	L	R	K	T	V	T	-	-	-	-	-	-	-	-	E	G	L	H	H	D	G	Q	F	C	H	K	P	C	P	P	G	E	R	K	A	R	D	C	T	V	FAS
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	H	Y	W	A	Q	G	K	L	C	C	Q	M	C	E	P	G	T	F	L	V	K	D	C	Q	CD27	
22	-	-	D	R	P	F	E	D	T	C	H	G	N	P	S	H	Y	Y	D	K	A	V	R	R	C	C	Y	R	C	P	M	G	L	F	P	T	Q	Q	C	P	Q	CD30
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K	Q	Y	L	-	-	I	N	S	Q	C	S	L	C	Q	P	G	Q	K	L	V	S	D	C	-	-	CD40
21	-	-	T	R	S	L	Q	D	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	S	N	C	P	A	G	T	F	-	-	-	C	D	N	4-1BB	
33	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	D	T	Y	P	S	N	D	R	-	C	C	H	E	C	R	P	G	N	G	M	V	S	R	C	S	R	OX40
28	-	-	P	N	G	K	C	K	D	T	E	Y	K	R	H	N	-	-	-	-	-	-	-	L	C	C	L	S	C	P	P	G	T	Y	A	S	R	L	C	D	S	VC22
26	-	-	S	N	G	K	C	K	D	N	E	Y	K	R	H	H	-	-	-	-	-	-	-	L	C	C	L	S	C	P	P	G	T	Y	A	S	R	L	C	D	S	CRMB
33	E	V	P	Q	Q	T	V	A	P	Q	-	-	-	-	-	Q	Q	R	H	S	F	K	G	E	E	-	-	-	C	P	A	G	S	H	R	-	-	-	-	-	TNFR-like	

FIG. 2B

75	P	-	G	Q	D	T	D	C	R	-	E	C	E	S	G	S	-	F	T	A	S	E	N	H	L	R	H	C	L	S	C	S	K	-	C	R	K	E	M	G	TNFR1	
68	T	K	T	S	D	T	V	C	-	D	S	C	-	E	D	S	T	Y	T	Q	L	W	N	W	V	P	E	C	L	S	C	G	S	R	-	C	S	D	Q	V	TNFR2	
60	N	Q	-	T	-	V	-	C	E	-	P	C	L	D	S	V	T	F	S	D	V	V	S	A	T	E	P	C	K	P	C	T	-	E	C	V	G	L	Q	S	NGFR	
73	S	R	S	Q	D	E	P	D	C	-	P	C	-	P	H	N	S	Y	N	E	H	W	N	H	L	S	T	C	Q	L	C	R	P	-	C	D	I	V	L	G	ITGR	
76	N	-	G	D	E	P	D	C	V	-	P	C	Q	E	G	K	E	Y	T	D	K	A	H	F	S	S	K	C	C	R	R	C	L	-	C	D	E	G	H	G	FAS	
56	H	R	K	A	-	A	Q	C	D	-	P	C	I	P	G	V	S	F	S	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CD27	
61	R	-	-	-	P	T	D	C	R	K	Q	C	-	E	P	D	Y	Y	L	D	E	A	D	R	C	T	A	C	V	T	C	S	-	-	-	-	R	D	D	CD30		
52	T	E	F	T	E	T	E	C	-	L	P	C	-	G	E	S	E	F	L	D	T	W	N	R	E	T	H	C	H	Q	H	K	Y	-	C	D	P	N	L	G	CD40	
40	N	R	N	Q	-	-	I	C	-	S	P	C	-	P	P	N	S	F	S	S	A	-	G	G	Q	R	T	C	D	I	C	R	-	Q	-	C	-	-	-	K	G	4-1BB
59	S	Q	N	T	-	V	-	C	R	-	P	C	-	G	P	G	F	Y	N	D	V	S	S	K	-	P	C	K	P	C	T	-	W	C	-	N	L	R	S	OX40		
60	K	T	N	T	-	-	Q	C	-	T	P	C	-	G	S	G	T	F	T	S	R	N	H	L	P	A	C	L	S	C	N	G	R	C	N	S	N	Q	V	VC22		
58	K	T	N	T	N	T	Q	C	-	T	P	C	-	A	S	D	T	F	T	S	R	N	H	L	P	A	C	L	S	C	N	G	R	C	D	S	N	Q	V	CFMB		
60	S	-	E	H	T	G	A	C	N	-	P	C	T	E	G	V	D	Y	T	N	A	S	N	N	E	P	S	C	F	P	C	T	V	-	C	K	S	D	-	-	TNFR-like	

FIG. 2C

6/23

111	-	Q	V	E	I	S	C	T	V	D	R	D	T	V	C	G	C	R	K	N	Q	Y	R	H	Y	W	S	E	N	L	F	Q	C	F	N	C	S	L	-	TNFR1	
106	-	-	E	T	Q	A	-	C	T	R	E	C	N	R	I	C	T	C	R	P	G	W	Y	C	A	L	S	K	Q	E	-	G	C	R	L	C	A	P	L	-	TNFR2
95	M	S	A	P	-	-	-	C	V	E	A	D	D	A	V	C	R	C	A	Y	G	Y	-	Q	D	E	T	T	G	-	R	C	C	E	A	C	R	V	-	NGFR	
110	F	E	V	A	P	-	C	T	S	D	R	K	A	E	C	R	C	Q	P	G	M	S	C	V	Y	L	D	N	E	-	-	C	V	H	C	E	E	-	LTBR		
113	L	E	V	E	I	N	-	C	T	R	T	Q	N	T	K	C	R	C	K	P	N	-	-	-	-	-	-	-	-	-	-	-	F	F	C	N	-	-	FAS		
74	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	H	H	T	R	P	-	H	C	E	S	C	R	H	-	CD27			
92	L	V	E	K	T	P	-	C	A	W	N	S	S	R	V	C	E	C	R	P	G	M	F	C	S	T	S	A	V	N	-	S	C	A	R	C	F	F	H	-	CD30
89	L	R	V	Q	Q	K	-	G	T	S	E	T	D	T	I	C	T	C	E	E	G	W	H	C	T	-	S	E	A	-	-	C	E	S	C	V	L	H	-	CD40	
71	V	F	R	T	R	K	E	C	S	S	T	S	N	A	E	C	D	C	T	P	G	F	H	C	L	-	-	G	A	-	G	C	S	M	C	E	Q	D	-	4-1BB	
92	G	S	E	R	K	Q	L	C	T	A	T	Q	D	T	V	C	R	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OX40		
96	-	-	E	T	R	S	-	C	N	T	T	H	N	R	I	C	E	C	S	P	G	Y	Y	C	L	L	K	G	S	S	-	G	C	K	A	C	V	S	Q	-	VC22
96	-	-	E	T	R	S	-	C	N	T	T	H	N	R	I	C	D	C	A	P	G	Y	Y	C	F	L	K	G	S	S	-	G	C	K	A	C	V	S	Q	-	CFMB
95	-	Q	K	H	K	S	S	C	T	M	T	R	D	T	V	C	Q	C	K	E	G	T	F	R	N	E	N	S	P	E	M	-	-	C	R	K	C	S	R	-	TNFR-like

FIG. 2D

TNFR-like

[illegible]

FIG. 2E

8/23

178	E	C	V	S	C	N	C	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	TNFR1					
178	I	C	R	P	H	Q	I	C	N	V	V	A	-	-	-	I	P	G	N	A	S	R	D	A	V	C	T	S	P	T	TNFR2			
163	P	C	L	P	C	T	V	C	E	D	T	E	R	Q	-	L	R	E	C	T	R	W	A	D	A	E	C	-	-	-	NFR			
186	R	C	Q	P	H	T	R	C	E	I	Q	G	L	V	E	A	A	P	G	T	S	Y	S	D	T	I	C	K	N	P	LTR			
142	H	C	D	P	C	T	K	C	E	-	-	-	-	-	-	-	-	-	-	-	-	H	G	I	-	-	I	K	E	C	FAS			
111	Q	C	K	D	-	K	E	C	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	C	-	-	D	P	L	CD27			
165	N	C	K	E	P	S	S	G	T	I	P	Q	-	A	K	P	T	P	V	S	P	A	T	S	S	A	S	T	M	P	V	CD30		
160	K	C	H	P	W	T	S	C	E	T	K	D	L	V	V	Q	Q	A	G	T	N	K	T	D	V	V	C	-	-	-	G	P	CD40	
133	-	C	R	P	W	T	N	C	S	L	D	G	K	S	V	L	V	N	G	T	K	E	R	D	V	V	C	G	P	S	P	-	-	4-1BB
140	A	C	K	P	W	T	N	C	T	L	A	G	K	H	T	L	Q	P	A	S	N	S	S	D	A	I	C	-	-	E	D	-	-	OX40
167	K	C	E	P	V	P	N	T	F	N	Y	I	D	V	E	I	T	L	Y	P	V	N	D	T	S	C	T	R	T	T	T	-	-	VC22
167	K	C	E	P	V	P	S	N	I	F	N	Y	I	D	V	E	I	N	L	Y	P	V	N	D	T	S	C	T	R	T	T	-	-	CRMB
140	N	C	T	S	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	D	I	Q	C	V	E	-	-	-	-	-	-	TNFR-like

FIG. 2F

9/23

[illegible]

FIG. 2G

SUBSTITUTE SHEET (RULE 26)

223	- - - - -	- - - - -	- - - - -	- - - - -	C L L S L L F I G L M Y - - - - -	T N F R 1
252	E G S T G D F A L - - - - -	- - - - -	- - - - -	P V G L I V G V T A L G L L I I G V V	T N F R 2	
236	M G S S Q P V V T R G T T D N L I P V Y C S I L A A V V V G L V A - - - - -	N G E R				
230	- - - - -	L - - - -	- - - - -	V L F L L F T T V L A C A W M R H P S	I T B R	
178	- - - - -	- - - - -	- - - - -	C L L - L L P I P L I V - - - - -	F A S	
164	A D - - - - -	- - - - -	- - - - -	- - - - -	C D 27	
242	K Q C E P D Y Y L D E A G R C T A C V S C S R D D L V E K T P C A W N S S R T C	CD30				
200	- - - - -	I - - - -	- - - - -	I F G I L F A I L L V L V F I K K - -	CD40	
190	- - - - -	F F L - - - -	- - - - -	- - - - -	4-1BB	
184	- - - A R P I T V Q P T E A - - - - -	- - - - -	- - - - -	- - - - -	O X 40	
221	P V F R E E Y - - - - -	- - - - -	- - - - -	F S V L N K V A T S G F F T G E N R	V C 22	
221	P V F R N G Y - - - - -	- - - - -	- - - - -	F S V L N E V A T S G F F T G Q N R	C R M B	
155	- - - - -	- - - - -	- - - - -	- - - - -	T N F R-like	

FIG. 2H

235	-	-	-	-	R	Y	Q	R	W	-	K	S	K	L	Y	S	I	-	-	-	-	V	C	G	K	S	T	P	E	K	E	G	L	E	G	T	TNFR1				
280	N	C	V	-	-	I	M	T	Q	V	K	K	P	L	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	TNFR2						
269	-	-	-	-	-	Y	I	A	F	K	R	W	N	S	C	K	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NGR						
250	L	C	R	-	-	K	L	G	T	L	L	K	R	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	LTR						
189	-	-	-	-	-	-	-	-	-	W	V	K	R	K	E	V	Q	K	-	-	-	-	T	C	R	K	H	R	K	E	N	Q	G	S	H	E	-	FAS			
166	-	-	-	-	-	-	-	-	-	F	R	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CD27						
282	E	C	R	P	G	M	I	C	A	T	S	A	T	N	S	C	A	R	C	V	P	Y	P	I	C	A	A	E	T	V	T	K	P	Q	D	M	A	E	K	D	CD30
218	-	-	-	-	-	-	-	-	-	-	V	A	K	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CD40				
193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4-1BB					
195	-	-	-	-	-	-	-	-	-	-	-	W	P	-	-	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	OX40					
246	-	-	-	-	-	Y	Q	N	I	S	K	-	-	V	C	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	VC22					
246	-	-	-	-	-	Y	Q	N	I	S	K	-	-	V	C	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CRMB					
155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	N	A	T	V	E	T	P	A	E	E	T	M	N	TNFR-like				

FIG. 21

12/23

264	T	K	P	L	A	P	N	P	S	F	S	P	T	P	G	F	T	P	T	L	G	F	S	P	V	P	S	S	T	F	T	S	S	T	Y	T	P	G	D	TNFR1	
294	-	-	-	-	-	-	-	L	Q	R	E	A	K	V	P	H	L	P	A	-	D	K	A	R	G	T	Q	G	P	E	Q	Q	H	L	I	T	A	-	-	TNFR2	
281	-	-	-	-	-	-	-	-	N	K	Q	G	A	N	S	R	P	V	-	N	Q	T	P	P	P	E	G	E	K	L	H	S	D	S	G	I	S	V	D	NFR	
262	-	-	-	-	-	-	-	-	P	E	G	E	S	P	C	P	A	-	P	R	A	D	P	H	F	P	D	L	A	E	P	L	-	-	-	-	-	-	LTR		
212	-	-	-	-	-	-	S	P	T	L	N	P	E	-	-	-	T	V	A	I	N	L	S	D	V	D	L	S	K	Y	I	T	-	-	-	-	-	-	FAS		
169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CD27			
322	T	T	F	E	A	P	P	L	G	T	Q	P	D	C	N	P	T	P	E	-	N	G	E	A	P	A	S	T	S	P	T	Q	S	L	L	V	D	S	Q	A	CD30
222	-	-	-	-	-	-	-	-	P	T	N	K	A	P	H	P	K	Q	E	-	P	Q	-	E	I	N	F	P	D	-	-	-	-	-	-	-	-	-	-	CD40	
193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4-1BB		
198	-	-	-	-	-	-	-	-	-	T	S	Q	G	P	S	T	R	P	V	-	E	-	-	-	V	P	G	G	R	A	V	A	A	I	L	G	L	G	L	-	OX40
255	-	-	-	-	-	-	-	-	L	N	F	E	I	K	C	N	N	-	-	-	-	-	-	K	G	S	-	-	S	F	K	Q	-	-	-	L	T	K	-	VC22	
255	-	-	-	-	-	-	-	-	L	N	F	E	I	K	C	N	N	-	-	-	-	-	-	K	D	S	Y	S	S	S	K	Q	-	-	-	L	T	K	-	CRMB	
170	T	S	P	G	T	P	A	P	A	E	E	T	M	N	T	S	P	G	T	P	A	P	A	E	E	T	M	T	T	S	P	G	T	-	-	-	-	-	-	TNFR-like	

FIG. 2J

304	C P N F A A P R R E V A P P Y Q G A D P I L A T A - - - - -	-	-	-	-	-	-	-	TNFR1
324	- - - - - P S S S S S L E S S A S A L - - - - -	-	-	-	-	-	-	-	TNFR2
311	S Q S L H D Q Q P H T Q T A S G Q A L K - - - G D G G L Y S S L P P A K R E E	-	-	-	-	-	-	-	NCR
288	- - - - - P M S G D L S P S P A G P P T - - - - -	-	-	-	-	-	-	-	LTR
236	- - - - - - - - - - - I A G V - - - - -	-	-	-	-	-	-	-	FAS
171	A R T L S T H W P P Q R S L C S S D F I - - - R I L V I F S G M F L V F T L A	-	-	-	-	-	-	-	CD27
361	S K T L P I P T S A P V A L S S T G K P V L D A G P V L F W V L V L V V G	-	-	-	-	-	-	-	CD30
241	- - - - - - - - - - - D L P G S N T A P V - - - - -	-	-	-	-	-	-	-	CD40
193	- - - - - - - - - - - A L T S T A L L F L - - - - -	-	-	-	-	-	-	-	4-1BB
224	- - - - - - - - - - - V L G L L - - - - - G P L A I L L A L Y L R R D Q	-	-	-	-	-	-	-	OX40
274	- - - - - A K N D D G - M - - - - -	-	-	-	-	-	-	-	VC22
276	- - - - - T K N D D S I - - - - -	-	-	-	-	-	-	-	CRM
205	- - - - - - - - - - - P A P A E E T M T T S - - - - -	-	-	-	-	-	-	-	TNFR-like

FIG. 2K

329	- - - L A S D P I P N P L Q K W E D S A H K P Q S L D T D D P A T L Y A V V E T N F R 1
339	- - - - - D R R A P T R N Q P Q A P G V E A S G A G E A R A S T G S S D S S T N F R 2
347	V E K L L - - - - - A P S L E E V L Q Q Q S P L - - - - - V Q A R E L - - - N G S N G F R
303	- - - - - M T L S Q V - - - - - - - - - - - - - - - - - E A E L I B R
240	- F A S
207	G A L F L - - - - - - - - - - - - - - - - - H Q - C D 27
401	S S A F L L C H R R A C R K R I R Q K L H L C Y P V Q T S Q P K L E L V D S R P C D 30
252	- - - - - Q E T L - - - - - - - - - - - - - - - - - C D 40
216	G R K K L L - - - - - - - - - - - - - - - - - 4-1B B
245	- O X 40
281	- - - - - - - - - - - M S H S E T V T L A G D C L S S V D I Y I L Y S N T N V C 22
284	- - - - - - - - - - - M P H S E S V T L V G D C L S S V D I Y I L Y S N T N C R M B
217	- - - - - P G T P A P A A E E T M T S P G T P A S - - - - - - - T N F R-like

FIG. 2L

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365	N	V	P	P	L	R	W	K	E	F	V	R	R	L	G	L	S	D	H	E	I	D	R	L	E	L	Q	N	G	R	C	L	R	E	A	Q	Y	S	M	L	TNFR1	
372	P	G	H	G	T	Q	V	N	V	T	C	I	V	N	V	C	S	S	D	H	-	S	S	Q	A	S	S	T	M	G	D	T	-	-	-	-	-	-	-	-	TNFR2	
355	A	G	D	T	W	R	H	L	A	G	E	L	G	Y	Q	P	E	H	I	D	S	F	T	H	E	A	C	P	V	R	-	-	-	-	-	-	-	-	-	-		
327	P	G	E	H	G	Q	V	A	H	G	A	N	G	I	H	V	T	G	S	V	T	V	T	G	N	I	Y	I	Y	N	G	P	V	L	G	G	T	-	-	-	-	NGFR
246	-	-	-	-	-	-	-	-	K	G	F	V	R	K	N	G	V	N	E	A	K	I	D	E	I	K	N	D	N	V	Q	D	T	A	E	Q	K	V	Q	L	L	LTbR
214	-	-	-	-	-	R	R	K	Y	R	S	N	K	G	E	S	P	V	E	P	A	E	R	C	R	Y	S	C	P	R	-	-	-	-	-	-	-	-	-	-	FAS	
441	R	R	S	S	T	Q	L	R	S	G	A	S	V	T	E	P	V	A	E	E	R	G	L	M	S	Q	P	L	M	E	T	C	H	S	V	G	A	A	Y	L	CD27	
256	-	-	-	-	-	-	-	-	H	G	C	Q	P	V	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CD30		
222	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CD40			
245	-	-	-	-	-	-	-	R	L	P	P	D	-	A	H	K	P	P	G	G	S	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4-1BB	
308	A	Q	D	Y	E	T	D	T	I	S	Y	R	V	G	N	V	L	D	D	S	H	M	P	G	S	C	N	I	H	K	-	-	-	-	-	-	-	-	-	-	OX40	
311	T	Q	D	Y	E	T	D	T	I	S	Y	H	V	G	N	V	L	D	V	D	S	H	M	P	G	R	C	D	T	H	K	-	-	-	-	-	-	-	-	-	VC22	
238	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CRMB		
																																									TNFR-like	

FIG. 2M

SUBSTITUTE SHEET (RULE 26)

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405	A T W R R R T P R R E A T L E L L G R V L R D M D L L G C - - - - -	-	-	-	-	-	-	-	TNFR1
409	- - - - - D S S P S E S - P K D E - - - Q V P F S K E E C A F - - -	-	-	-	-	-	-	-	TNFR2
388	A S W A T Q D S A T L D A - - - - -	-	-	-	-	-	-	-	NGFR
365	- - - - - R G P G D P - P A P P - - - E P P Y P T P E E G A - - -	-	-	-	-	-	-	-	LTDK
279	R N W H Q L H G K K E A - Y D T L I K D L K K A N L - - C T L A E K I Q - - -	-	-	-	-	-	-	-	FAS
239	- - - - - E E E G S T I - - - - -	-	-	-	-	-	-	-	CD27
481	E S L P L Q D A S P A G G - P S S P R D L P E P R V S T E H T N K I E K I Y I	-	-	-	-	-	-	-	CD30
263	- - - - -	-	-	-	-	-	-	-	CD40
222	- - - - -	-	-	-	-	-	-	-	4-1BB
260	- - - - -	-	-	-	-	-	-	-	OX40
339	- - - - -	-	-	-	-	-	P I T N S K - - - - -	-	VC22
342	- - - - -	-	-	-	-	-	L I T N S N S Q Y - - - - -	-	CRMB
238	- - - - -	-	-	-	-	-	S H Y L S C T I V G I V - - - - -	-	TNFR-like

FIG. 2N

[illegible]

FIG. 20

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[illegible]

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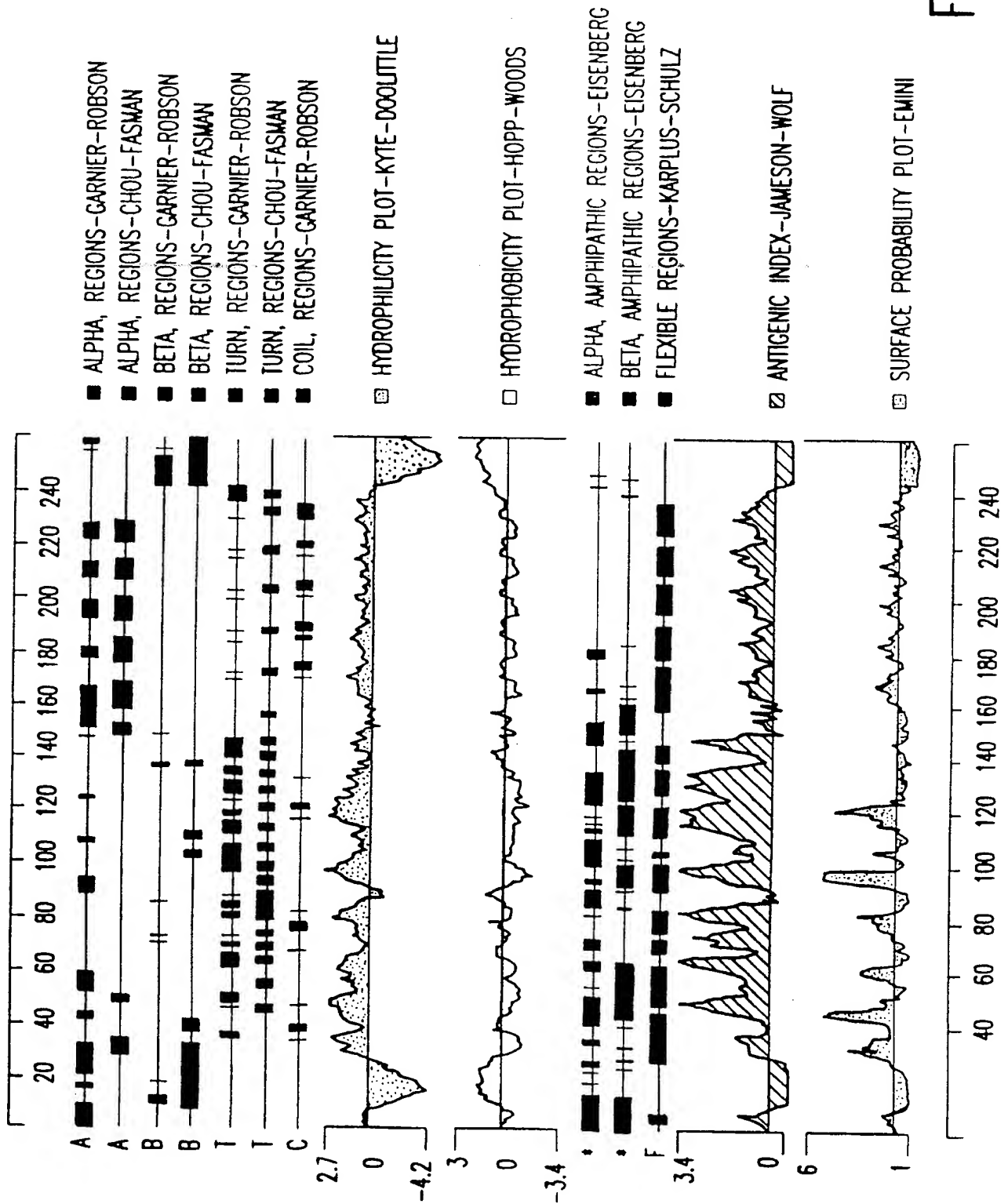


FIG.3

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HPRCB54R

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1   GAATTCGGCA NAGCCTCTCC ACGCGCACGA ACTCAGCCAA CGATTTCTGA
51  TAGATTTTGG GGAGTTTGAC CAGAGATGCA AGGGGTGAAG GAGCGCTTCC
101 TACCGTTAGG AACTCTGGGG ACAGNNCGCC CCGGCCGCCT GATGGCCGAG
151 GCAGGGTGCG ACCCAGGACC CAGGACGGCG TCGGGAACCA TACCATGGCC
201 CGGATCCCCA AGACCCTAAA GTTCGTGGTC GTCATCGTCG CGGTCCTGCT
251 GCCAGTCCTA GCTTACTCTG CCACCACTGC CCGGCAGAGG AAGTTNCCCA
301 GCAGNCANTG GNCCCACAGC AACAGNGGCA CAGTTTCAAG GGGGNAGGAG
351 TTTTCCANCA AGTTTTTATA GTTCAGAACN TATTGGNGCT NTNAACCCTT
401 GCACAAGGGT TTGGNTTAAA CCAANGTTTC CAANATGNAC TTTTNGTTT
451 CCTGTTANAT TTTTAAATTA GTTNAAANTT AAATTTNTNA ACCTTNCCNG
501 GGNAAATT

```

HSJAU57RA

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1   GGCAGAGGTG TCTCCAGCCT GGCTCTATCT TCCTCCTTGT NATCGTCCCA
51  TCCCCACATC CCGTGCACCC CCCAGGACCC TGGTCTCATC AGTCCCTCTC
101 CTGGAGCTGG GGGTCCACAC ATCTCCCAGC CAAGTCCAAG AGGGCAGGGC
151 CAGTTCCTCC CATCTTCAGG CCCAGCCAGG CAGGGGGCAG TCGGCTCCTC
201 AACTGGGTGA CAAGGGTGAG GATGAGAAGT GGTACGGGG ATTTATTAG
251 CTTGGTCAG AGCAGAACAC AGAGTTTTTC CGTGTGTTGG TTTTACTCT
301 NNTTCCCCTT CTTNATNCCC CTTTCN

```

HUSCB54R

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1   CCAGGGTCTC CTNCCCCACC TGCTGAAGAG ACANTGACCA CCAGCCCGGG
51  GACTCCTGCC TCTTCCTCAT TACCTCTNAT GNANCATCGT AGGGATCATA
101 GTTCTAATTG TGCCTTCTGA ATTGTGCTTT GTTTGGAAAG ACTTCACTGT
151 GGAAGAAAT TCCTTCCTTA CCTGAAGTTG CAGGTAGGCC CTGGGTNAGG
201 GCGNGGGGCG CTGGACANTN TCTGGNCCTG GCTGCCCCGCT G

```

HELBP70R

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1   GGCAGAGGCC CCAGCTGCTG AAGAGACAAT AATCACCAGC CCGGGGACTC
51  CTGNNTCTNC TNATTACCTC TNATGCACCA TCGTAGGGAT CATAGTTCTA
101 ATTGTGCCTT CTAATTGTTT TTGTTTGAAA AGANTTCACT GTGGAAGAAA
151 TTCCTTCCTT ACCTGTAAGT TNCAGGTAGG NGCCTGGCTG AGGGCGGGGG
201 GCGCTGGTAC ACTCTCTGAC CCTGCCTCCC TCTGNCTGTT TTCCACAGA
251 CAGAAACGCC TGCCCCTGNC CCAAGTTCC TNGTGTTTTT CAGCCTGGCT
301 CTATCTTNNC TCCTTG TGAA TCGTTCCCAT CCCCACANGC

```

FIG.4

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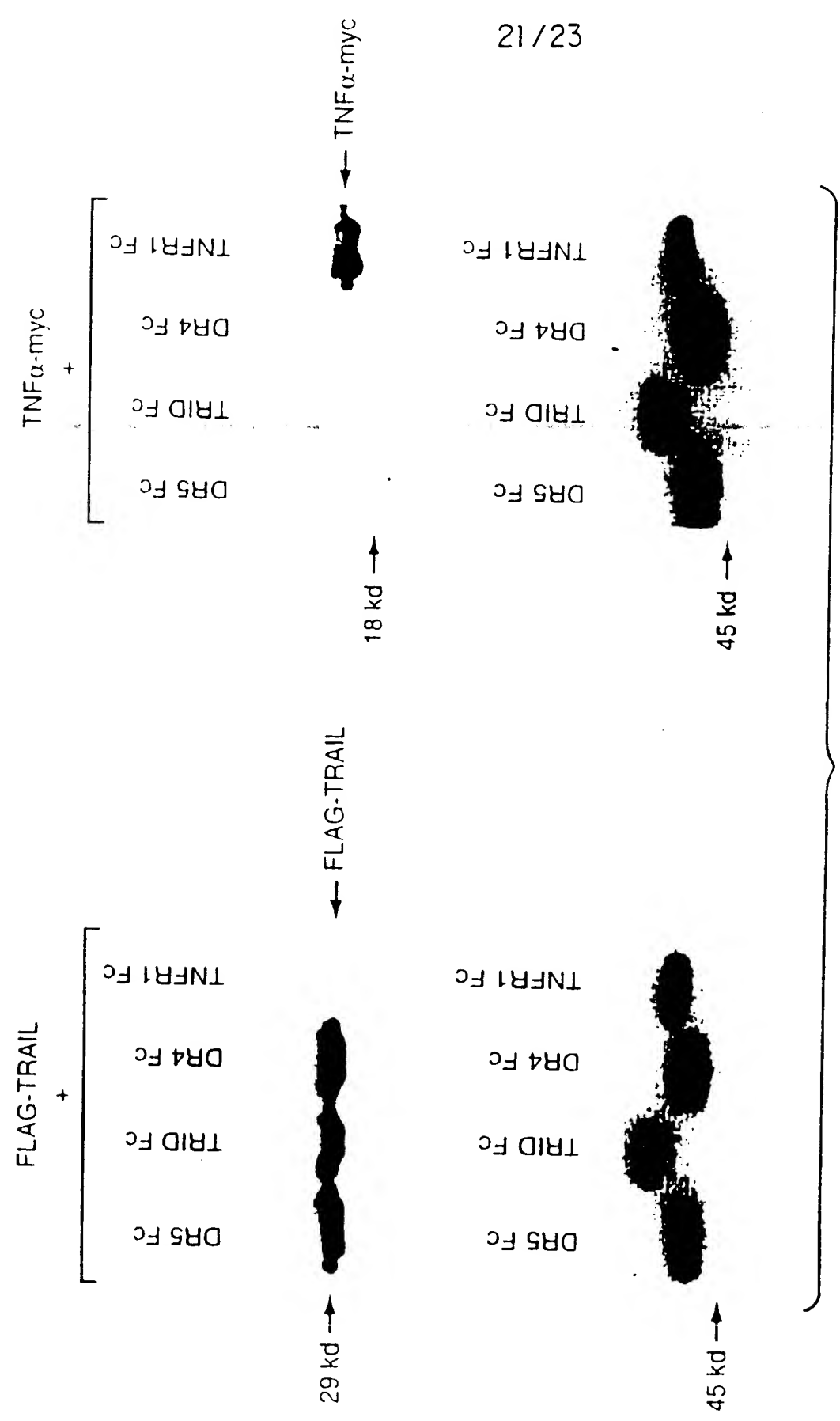


FIG. 5A

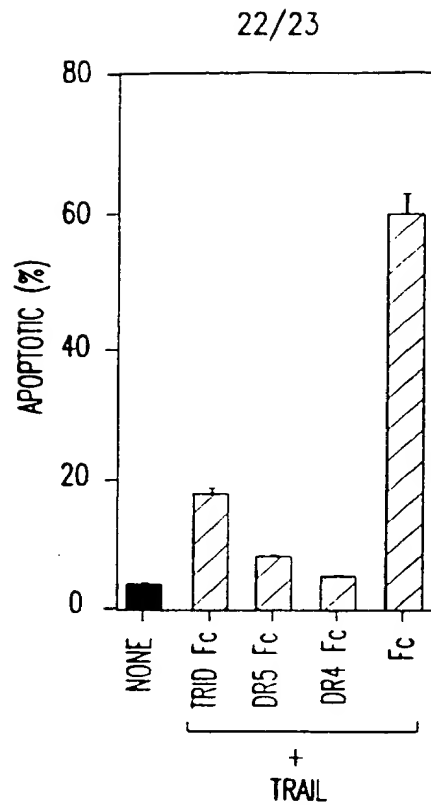


FIG.5B

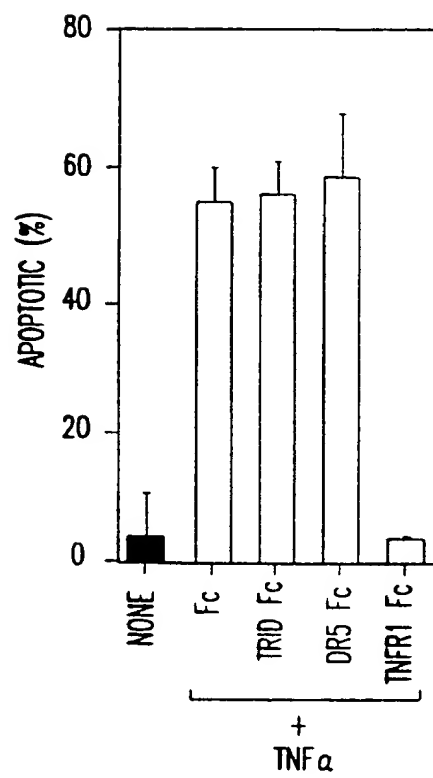


FIG.5C

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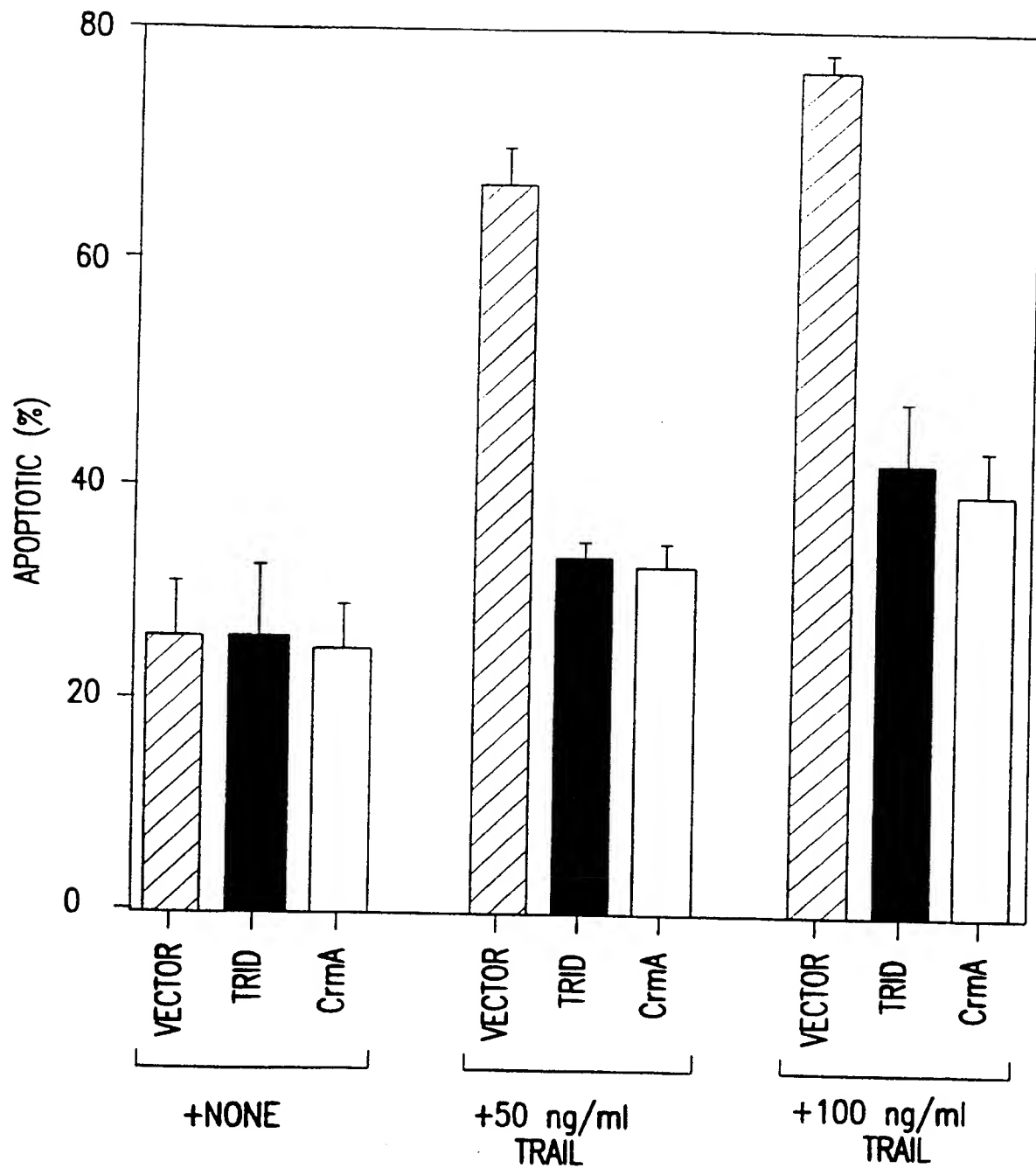


FIG.6

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

Intern: al Application No

PCT/US 98/00152

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/70 C12N15/85 C12N15/86 C12N1/21
 C12N5/10 C07K14/705 C07K16/28 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PITTI R.M. ET AL.: "Induction of apoptosis by Apo-2L, a new member of the tumor necrosis factor cytokine family" J. BIOL. CHEM., vol. 271, no. 22, 31 May 1996, pages 12687-12690, XP002065021 cited in the application see abstract see page 12690, column 1, line 8 - line 24 ---	1-22
P,X	PAN G. ET AL.: "An antagonist decoy receptor and a death domain-containing receptor for TRAIL" SCIENCE, vol. 277, - 8 August 1997 pages 815-818, XP002065147 see the whole document --- -/-	1-22



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

19 May 1998

Date of mailing of the international search report

03.07.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Galli, I

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SHERIDAN J.P. ET AL.: "Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors" SCIENCE, vol. 277, 8 August 1997, pages 818-821, XP002065023 see the whole document ---	1-22
P,X	MACFARLANE M. ET AL.: "Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL" J. BIOL. CHEM., vol. 272, no. 41, 10 October 1997, pages 25417-25420, XP002065148 see abstract see figure 1B ---	1-22
P,X	SCHNEIDER P. ET AL.: "Characterization of two receptors for TRAIL" FEBS LETTERS, vol. 416, 27 October 1997, pages 329-334, XP002065022 see the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/00152

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 22 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

